AMPHIBIAN CONSERVATION IN 3D:
DISEASE, DIVERSITY, AND DEFORESTATION

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AMPHIBIAN CONSERVATION IN 3D:
DISEASE, DIVERSITY, AND DEFORESTATION

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Habitat loss and chytridiomycosis (a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* - *Bd*) are major drivers of amphibian declines worldwide. Understanding how their independent and interactive effects lead to amphibian declines is critical for biodiversity conservation. For my doctoral research, I (1) developed a novel integrative approach for systematic conservation planning for tropical amphibians facing negative impacts of accelerated habitat loss, (2) quantified the influence of deforestation on the risk of chytridiomycosis, and (3) through a combination of field surveys and laboratory-controlled experiments, identified abiotic and biotic mechanisms that link deforestation to shifts in disease dynamics. Chapter 1 evaluates different scenarios of systematic conservation planning for Brazilian amphibians, integrating data on species life-history and ecologically relevant spatial metrics of deforestation and landscape configuration. Chapter 2 describes a paradoxical negative relationship between habitat loss and the risk of chytridiomycosis in amphibian populations from Costa Rica, Brazil, and Australia, laying the foundation for chapters 3, 4, and 5, which focus on mechanisms by which deforestation alters disease dynamics. Chapter 3 describes cascading effects of deforestation linking reduction in canopy cover to microclimatic shifts, which affect both *Bd* prevalence and infection intensity. Chapter 4 experimentally tests and finds support for the 'dilution effect' hypothesis, which predicts an inverse relationship

between host diversity and disease. In Chapter 5, I show that temperate amphibian communities with higher population densities at pristine closed-canopy sites carry higher *Bd* loads due to density-dependent transmission. In contrast, tropical amphibian communities commonly found in pristine forests carry lower *Bd* infection loads than those in disturbed habitats, presumably due to their host species composition. These results combined highlight that deforestation shifts abiotic and biotic factors, which in turn can either increase or decrease disease risk and impact conservation efforts in amphibians. My dissertation work has important analytical and theoretical implications for the field of disease ecology and the management of amphibians facing the dual negative impacts of habitat loss and chytridiomycosis.

BIOGRAPHICAL SKETCH

Carlos Guilherme Becker (Gui) was born and raised in Salvador do Sul, a very small town in the State of Rio Grande do Sul, Southern Brazil. Gui pursued an undergraduate degree in Biological Sciences at University of Vale do Rio dos Sinos in São Leopoldo, Southern Brazil. He majored in Biology with an emphasis in Ecology & Education and spent two years investigating the impacts of exotic silvicultures on the population dynamics of amphibians of the Araucaria Moist Forest. After completing his undergraduate degree, Gui moved to São Paulo where he attained his Master's degree at State University of Campinas. He majored in Ecology and spent two years investigating how discontinuity between terrestrial and aquatic habitats impacts migration in amphibians from Brazil's Atlantic Forest. He has been interested in how destruction of natural habitats influences ecological and evolutionary processes ever since.

From 2008 to 2014 he worked with Dr. Kelly R. Zamudio at Cornell University studying the mechanisms by which deforestation impacts the spread of the frog-killing fungus *Batrachochytrium dendrobatidis* in tropical and temperate regions. His research interests now fall at the interface of spatial ecology and wildlife epidemiology, with detailed mechanistic studies of diversity-disease relationships, host-pathogen responses to abiotic factors, connectivity and dispersal of animal populations. Upon finishing his degree at Cornell University, Gui will move to Brazil to begin a junior researcher position in the State University of São Paulo, in the

laboratory of Dr. Célio Haddad, where he will continue studying spatial epidemiology in amphibians. Specifically, his future research program will focus on integrating the study of host-pathogen interactions with spatial analyses of genotypes and phenotypes, to provide a mechanistic understanding about how biodiversity loss and genetic erosion influence the risk of epidemics, and how combining both kinds of data will inform efficient strategies for biodiversity conservation.

Dedicated to

my wife, Mônica,

for her love and support.

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I owe a great deal of thanks to many people that have supported me during my tenure at Cornell. Dr. Kelly Zamudio has given me incredible mentorship on how to be a scientist and critical thinker. She is an outstanding mentor, friend, and collaborator that will do anything for her students to succeed both academically and in life. Dr. Alexander Flecker laid the foundation for my understanding of ecological processes driving wildlife diseases. Dr. Harry Greene has given me advice on fundamental topics of ecology and evolutionary biology, always reminding me of the importance of natural history for evolutionary ecology. Dr. Anurag Agrawal has helped me with important aspects of experimental design and data analysis. I am also grateful to have served as a graduate teaching assistant for Kelly, Alex, Harry and Anurag. Their engaging teaching philosophy fostered thought-provoking discussions and completely changed the way I teach.

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I thank Mônica for all the great things I have in my life. She also helped with experimental design and data analyses improving several chapters in this dissertation. Samuel brought a new powerful energy to my life. Both have been the joy of my life and will continue to be my source of motivation.

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CHAPTER 1

INTEGRATING SPECIES LIFE-HISTORY TRAITS AND PATTERNS OF DEFORESTATION IN AMPHIBIAN CONSERVATION PLANNING

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Abstract

Aim: To identify priority areas for amphibian conservation in southeastern Brazil, by integrating species life-history traits and patterns of deforestation.

Location: State of São Paulo, Brazil.

Methods: We used the software Marxan to evaluate different scenarios of amphibian conservation planning. Our approach differs from previous methods by explicitly including two different landscape metrics; habitat split for species with aquatic larvae, and habitat loss for species with terrestrial development. We evaluated the effect of habitat requirements by classifying species breeding habitats in five categories (flowing water, still water permanent, still water temporary, bromeliad or bamboo, and terrestrial). We performed analyses using two scales, grid cells and watersheds and also considered nature preserves as protected areas.

Results: We found contrasting patterns of deforestation between coastal and inland regions. Seventy-six grid cells and 14 watersheds are capable of representing each species at least once. When accounting for grid cells already protected in state and national parks and considering species habitat requirements we found 16 high-priority grid cells for species with one or two reproductive habitats, and only one cell representing species with four habitat requirements. Key areas for the conservation of species breeding in flowing and permanent still waters are concentrated in southern state, while those for amphibians breeding in temporary ponds are concentrated in central to eastern zones. Eastern highland zones are key areas for preserving species breeding terrestrially by direct or indirect development. Species breeding in bromeliads and bamboos are already well represented in protected areas.

Main conclusions: Our results emphasize the need to integrate information on landscape configuration and species life-history traits to produce more ecologically relevant conservation strategies.

Introduction

Deforestation does not progress randomly throughout landscapes (Viana et al., 1997; Ezard & Travis, 2006; Silva et al., 2007) because of the non-random spatial distribution of land suitable for specific human activities and laws that often protect particular habitats or vegetation types. Riparian vegetation, for example, is protected by law in many countries (Gregory et al., 1991). Thus, if enforcement is effective, deforestation should be biased to areas other than riparian zones. In contrast, humans tend to concentrate their activities in valleys where water is readily available for agriculture, industry and human consumption, resulting in deforestation and disconnection of riparian zones from upland vegetation (Viana et al., 1997; Silva et al., 2007). Wetter parts of the landscape, which encompass a large proportion of amphibian breeding sites, are frequently converted for agricultural or urban development (Viana et al., 1997).

Amphibian species with different developmental modes vary in their responses to habitat change (Gascon et al., 1999; Tocher et al., 2001; Bell & Donnelly, 2006; Urbina-Cardona et al., 2006; Becker et al., 2007). Species with aquatic larvae often need landscape complementation, relying on the integrity of and connection between terrestrial and aquatic habitats to complete their biphasic life cycles (Werner & Gilliam, 1984; Pope et al., 2000; Becker et al., 2010). Discontinuity between suitable

aquatic and terrestrial habitats forces many species with aquatic larvae to perform risky breeding migrations through disturbed environments, potentially contributing to population declines (Becker et al., 2010). This habitat split is the strongest factor determining richness of amphibians with aquatic larvae in the Brazilian Atlantic Forest (Becker et al., 2007). Conversely, most species with terrestrial development can complete their life cycle in the absence of water bodies (Haddad & Prado, 2005) and thus suffer primarily with the loss of terrestrial vegetation. Therefore, distinct patterns of deforestation have different effects on the spatial configuration of terrestrial and aquatic habitats, and these, in turn, can affect amphibian species with distinct life-history traits in different ways (Becker et al., 2007).

The Brazilian Atlantic Forest is a biodiversity hotspot, harbouring a large number of endemic frogs (Morellato & Haddad, 2000; Mittermeier et al., 2005). This biome is threatened because of severe habitat destruction, and the spatial distribution of upland forests and lowland riparian vegetation varies across regions. Therefore, areas with the same overall deforestation rate can have very different degrees of natural riparian vegetation remaining, as well as varying connectivity with terrestrial environments (Rodrigues et al., 2009). This variation makes this region an ideal place to investigate how species life-history traits interact with deforestation patterns, and the implications for amphibian conservation planning. In addition, the conflict between conservation of water resources and destruction of terrestrial vegetation in riparian zones is a key factor in amphibian conservation planning, yet its effects on amphibian communities have not yet been fully explored (Becker et al., 2010).

Here, we apply conservation planning analyses to southeastern Brazil to identify sets of priority areas for amphibian conservation, considering the current network of protected areas in the State of São Paulo, Brazil. Our ultimate goal is to pinpoint areas for habitat protection and inventory work before we lose a considerable portion of amphibian diversity. Our approach differs from previous methods by explicitly including different landscape metrics – habitat split, for species with aquatic larvae, and habitat loss, for species with terrestrial development – to develop conservation planning scenarios. We tested the effect of habitat requirements classifying species breeding habitats in five categories [flowing water, still water permanent, still water temporary, bromeliad and bamboo, and terrestrial (e.g., leaf litter, soil)]. We suggest alternative habitat restoration plans according to species life-history requirements.

Methods

Study regions

Originally extending for 1,300,000 km^2 along the Brazilian coast and into parts of Paraguay and Argentina, the Brazilian Atlantic Forest has been reduced to c. 10% of its historical range (Morellato & Haddad, 2000; Rodrigues et al., 2009). Fragments currently remaining harbour a fauna and flora with one of the highest levels of endemicity in the world, with many species of vertebrates still being described (Alves et al., 2009; Pimenta et al., 2009). For amphibians in particular, the Atlantic Forest harbours c. 300 endemic species, many of which have suffered population declines and local extinctions attributed primarily to habitat change (Heyer et al., 1988; Weygoldt,

1989; Eterovick et al., 2005; Becker et al., 2007). Moreover, in some regions, patterns of human economic development have resulted in landscapes with a large number of upland 'dry fragments', disconnected from streams and other water sources at lower elevations. Other regions, however, still have intact riparian buffers despite substantial deforestation (Viana et al., 1997; Silva et al., 2007).

We focused on the State of São Paulo, which was historically dominated by Atlantic Forest with some remnants of Cerrado in the central and westernmost parts of the state (Ribeiro et al., 2009). São Paulo is the most populated state in Brazil, with c. 40 million inhabitants. Over the last century, massive deforestation for agriculture, cattle-raising and urbanization (Viana et al., 1997; Morellato & Haddad, 2000) has confined many remnants of natural vegetation to steep slopes and hilltops (Viana et al., 1997; Silva et al., 2007). Currently, the remaining fragments of natural vegetation are concentrated in the coastal zones (Instituto Florestal, 2004; Ribeiro et al., 2009).

Data

We gathered a database of 220 anuran species known from the State of São Paulo (Araújo et al., 2009) and compiled their respective geographic ranges (IUCN et al., 2009). Using GIS procedures in Arc View 9.3 (ESRI, 2008), we divided the region into (1) a regular equal area grid of 220 cells (hexagons of 1200 km^2) and (2) 22 watersheds (mean watershed size: 11273.9 km^2 ± 5451.1 SD). Species geographic ranges were overlaid to extract data on species richness for each grid cell and/or watershed. We chose these two different spatial approaches to explore how the choice of uniform or topographically relevant spatial limits affects the outcome of

conservation planning. In particular, grid cells are spatially consistent, but random relative to the environment, and each identifies diversity at a small spatial scale. In contrast, watersheds are regionally bound due to topography, and thus may better reflect biogeographical assemblages of species but also usually encompass a much larger area.

We obtained a GIS database (1:50,000) with information on land cover, watershed and protected areas (IUCN categories I–IV: ecological stations, state and national parks) from the Programa Biota and Instituto Florestal, an initiative of the Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Instituto Florestal, 2004). We obtained the state hydrological database (1:50,000) from the Instituto Brasileiro de Geografia e Estatística – IBGE (IBGE, 1985).

Analyses

To identify priority areas for amphibian conservation, we grouped species by their developmental mode: species with aquatic larvae (176 species) or species with terrestrial development (44 species). The determination of developmental mode followed previously defined categories of reproductive types identified for Neotropical anurans (Haddad & Prado, 2005). Species with developmental modes requiring aquatic habitats (running and still surface waters) were classified as species with aquatic larvae, whereas species completing their development in terrestrial vegetation (leaf litter, soil, bromeliad tanks and bamboo stems) were classified as species with terrestrial development (see also Loyola et al., 2008a). We classified species breeding habitats in five categories: flowing water (FW), still water/permanent (SP) and still

water/temporary (ST) for species with aquatic larvae; and bromeliad or bamboo (BB), and terrestrial (DI) (e.g., direct and indirect terrestrial developers) for species with terrestrial development. The reason we considered species breeding in bromeliad tanks or bamboo stems as terrestrial developers is because these species rely on terrestrial vegetation independent of the presence of surface waters (Haddad & Prado, 2005).

We used the software Marxan (v. 1.8.2) to evaluate and compare different conservation planning scenarios (Possingham et al., 2000). Using an optimization procedure, based on a simulated annealing algorithm, we identified priority sets of areas (grid cells or watersheds) that represent, as a set, all species at least once, based on the complementarity concept (Kirkpatrick, 1983; Pressey et al., 1996). This algorithm begins with a random set of grid cells/watersheds, and at each iteration, swaps units in and out of that set, measuring the change in cost according to a cost function. The procedure was repeated 100 times, and final conservation planning scenarios (i.e. sets of grid cells or watersheds) were obtained after 10 million iterations. Marxan allows users to set a penalty value for losing a species. We set a high penalty value to ensure that all species were represented with a minimum number of grid cells or watersheds. As each Marxan run provides a somewhat different solution, we used the metric 'selection frequency' to compare scenarios. Selection frequency represents the number of times each planning unit is selected in the solutions to the overall problem (Leslie et al., 2003). Planning units (here grid cells and watersheds) that are selected above a certain threshold-percentage (>90%) of runs are considered as high priority conservation areas.

Several spatial prioritization studies aim to meet predefined conservation objectives for the minimum total 'cost'. Conventionally, the cost of a site for conservation is simply proportional to its area. Here, we examined the cost of the grid cells or watersheds selected by the optimization algorithm based on two 'cost' metrics: habitat split and habitat loss. Thus, each grid cell and watershed received a 'cost' value based on current values of these landscapes metrics. Habitat split was calculated as the percentage of total stream length and permanent pond perimeter that did not overlap with natural forest cover within each grid cell or watershed. Habitat loss was the percentage of non-natural vegetation cover occurring within each grid cell or watershed. Because amphibians with aquatic larvae are expected to have a stronger response to habitat split, and species with terrestrial development to habitat loss (Becker et al., 2007), we used these costs as constraints on the analyses.

Sets of priority areas capable of representing species with aquatic larvae (including FW and SP reproductive habitats) should, whenever possible, favour the inclusion of grid cells or watersheds with lower values of habitat split. Conversely, key areas for representing species with terrestrial development (including BB and DI reproductive habitats) should favour the inclusion of grid cells or watersheds with the lowest values of habitat loss. Because temporary ponds (ST) are not identifiable in the hydrology maps, and can also be associated with upland habitats, we set equal constraints of habitat split and habitat loss for species using this breeding habitat. Areas with high selection frequency in our analyses were designated as the highest priority set. Sets of grid cells or watersheds obtained from these analyses were drawn on grid or watershed maps using ArcView GIS 9.3 (ESRI, 2008). We combined maps

to reveal complementary sets of areas with distinct values of selection frequency that should be included in a reserve system to represent all species with aquatic larvae and those with terrestrial development.

The State of São Paulo already has several protected natural areas. To evaluate their effectiveness and quantify how much additional area needs to be preserved to safeguard the state's amphibian diversity, we ran additional analyses considering grid cells including protected areas (IUCN categories I–IV and larger than 300 ha). These cells (n = 56) were fixed in the analyses because they are already protected. This procedure, often referred to as a 'gap analysis' (Margules & Pressey, 2000), allowed us to identify new grid cells that best complement the current reserve network established in the state. Values of habitat split and habitat loss were also used as constraints in these analyses, according to species reproductive habitats, as described above. We used UTM WGS 1984 projection for maps resulting for all analyses.

Finally, we tested whether including amphibian life-history traits into conservation planning actually changes or improves the selection process. Recently, we demonstrated that the ideal choice of priority areas for amphibian conservation depends on their developmental modes (Loyola et al., 2008a), but that study did not include both cost metrics (habitat split and habitat loss). Here, we selected priority areas based on both cost metrics and also, without considering differences in such life-history trait. For this last analysis we pooled species with aquatic larvae and terrestrial development into other Marxan runs (performed in the same way as described above). We then compared the total cost of conservation planning scenarios, based on total habitat split and habitat loss found across the selected grid cells. We tested differences

of mean habitat split and habitat loss among conservation scenarios that separated species by developmental mode and considering all species together. In this analysis, we used grid cells with high selection frequency (>90%) and applied arcsine-square root data transformation to perform this test.

Results

Patterns of deforestation and species richness

We found contrasting patterns of deforestation in the coastal and inland regions of São Paulo State (Figure 1.1). Deforestation in coastal and more densely populated regions is less severe than that observed in inland agricultural zones; however, deforestation in coastal regions is biased to riparian vegetation, leading to high habitat split at the landscape level, and affecting primarily amphibians with aquatic larvae. Inland agricultural zones, despite having higher rates of habitat loss than coastal areas, harbour more riparian vegetation than expected by chance. This result holds for grid cell (Figure 1.1a) and watershedbased analyses (Figure 1.1b).

We found a gradient in species richness increasing toward eastern São Paulo both for species with aquatic larvae (Figure 1.2a-b) and terrestrial development (Figure 1.2c-d). This gradient is consistent across grid cell and watershed analyses. A single grid cell showed higher richness for both amphibians with aquatic larvae (91 species; 52% of the total) and terrestrial development (24 species; 55% of the total); this cell is located in the eastern coastal region of the state (highlands of Serra da Bocaina, Vale do Paraíba watershed) (Figure 1.2).

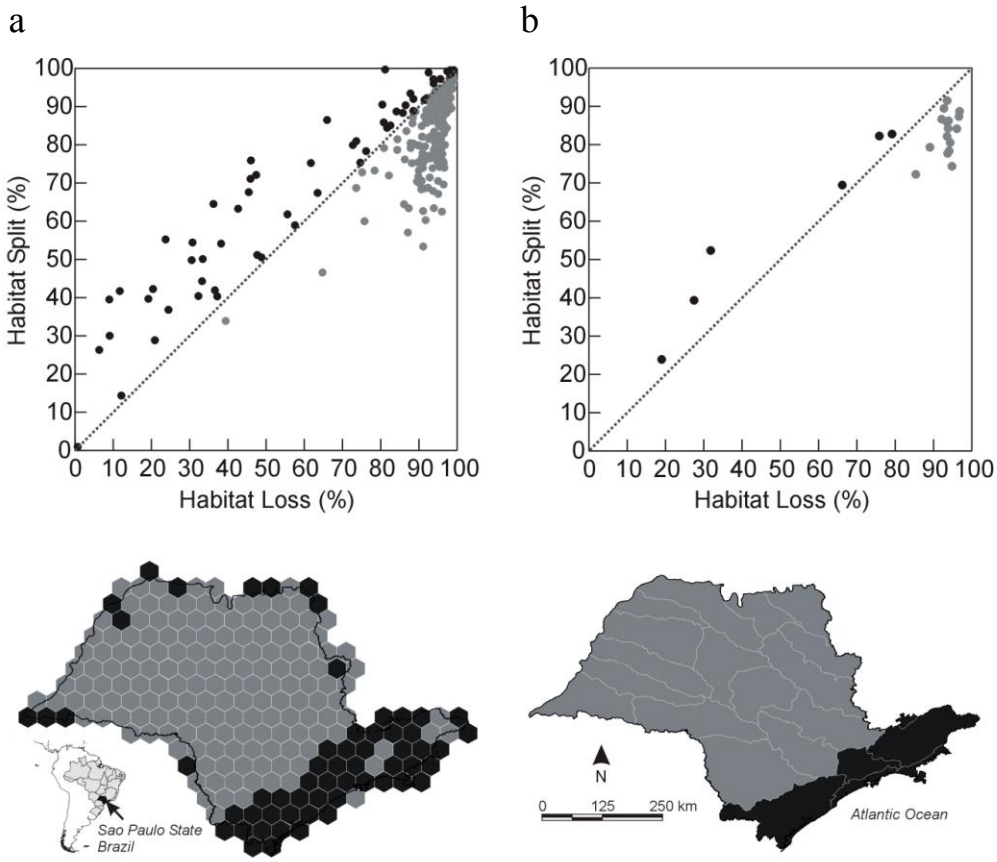

Figure 1.1: Relationship between habitat loss and habitat split for grid cells (a) and watersheds (b). Dotted line represents expected values if deforestation occurred randomly throughout the landscape. Black represents deforestation biased towards riparian buffers. Grey represents deforestation biased to non-riparian parts of the landscape.

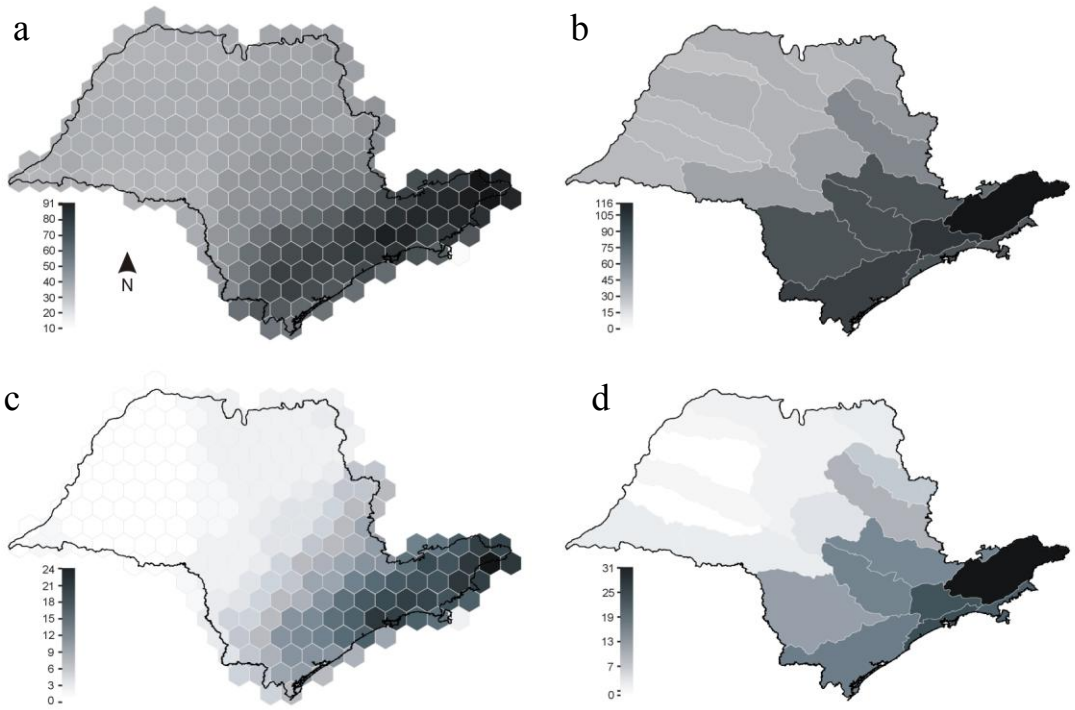

Figure 1.2: Patterns of species richness for species with aquatic larvae (a, b) and terrestrial development (c, d). Scale represents number of species.

Priority areas for amphibian conservation

When amphibian developmental modes were not taken into account (all species pooled), the mean habitat split and habitat loss found across priority grid cells was higher (Figure 1.3). Priority areas inferred from independent analyses considering species with aquatic larvae and terrestrial development yielded lower levels of habitat split (t-test, $t = 2.417$, d.f. = 27, $P = 0.023$) and habitat loss ($t = 2.150$, d.f. = 17, $P = 0.046$) respectively. Hence, conservation planning scenarios that ignore amphibian reproductive modes did not reduce habitat split and habitat loss in the inferred protected areas compared with independent analysis including life-history traits (Figure 1.3).

When amphibian reproductive habitats were included as ecological constraints into spatial prioritization analyses we found 76 grid cells and 14 watersheds capable of representing each species at least once (Figure 1.4). Grid cells and watersheds with high selection frequency for preserving species that breed in flowing waters were concentrated in coastal areas and northeastern São Paulo state (Figure 1.4a-b). Grid cells crucial to preserving species that breed in still permanent (Figure 1.4c-d) and temporary water bodies (Figure 1.4e-f) were scattered across the entire state. Species breeding in bromeliad tanks and bamboo stems can be safeguarded by protecting central to northern coastal region (Figure 1.4g-h). Species with direct or indirect terrestrial development will be most protected by preserving grids in eastern parts of the state and some isolated areas in the northeast (Figure 1.4i-j). These results were independent of the scale of analyses.

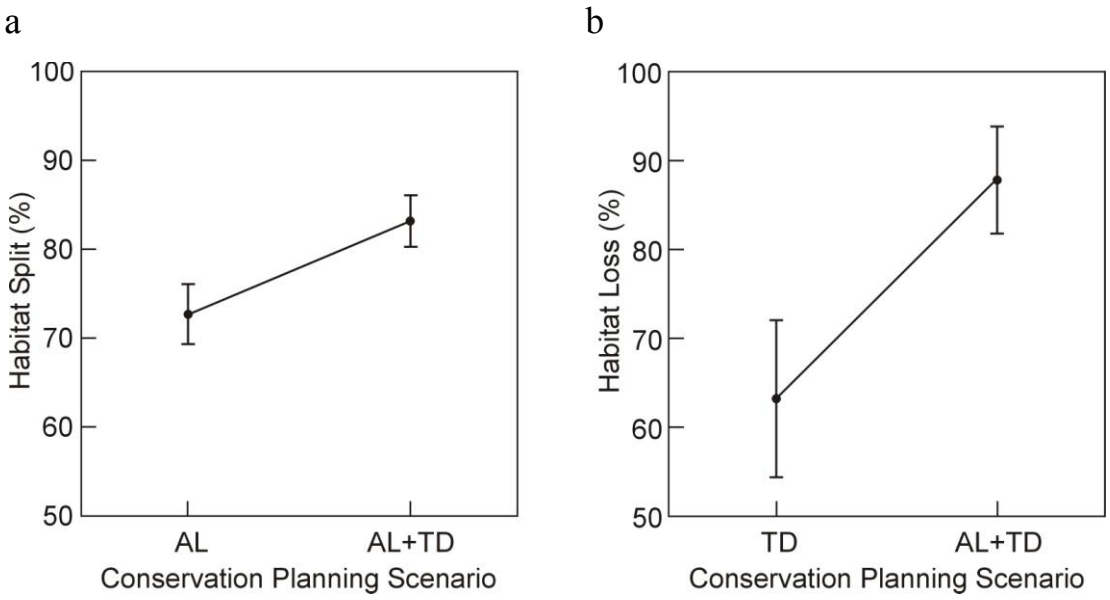

Figure 1.3: (a) Mean habitat split found within high-priority grid cells (>90% selection frequency) under two conservation planning scenarios; AL considered only species with aquatic larvae; AL + TD considered all species at the same time. All conservation planning scenarios aimed at minimizing habitat split within the landscape. (b) Mean habitat loss found within high-priority grid cells under two conservation planning scenarios; TD considered only species with terrestrial development; AL + TD considered all species at the same time. All conservation planning scenarios aimed at minimizing habitat loss within the landscape.

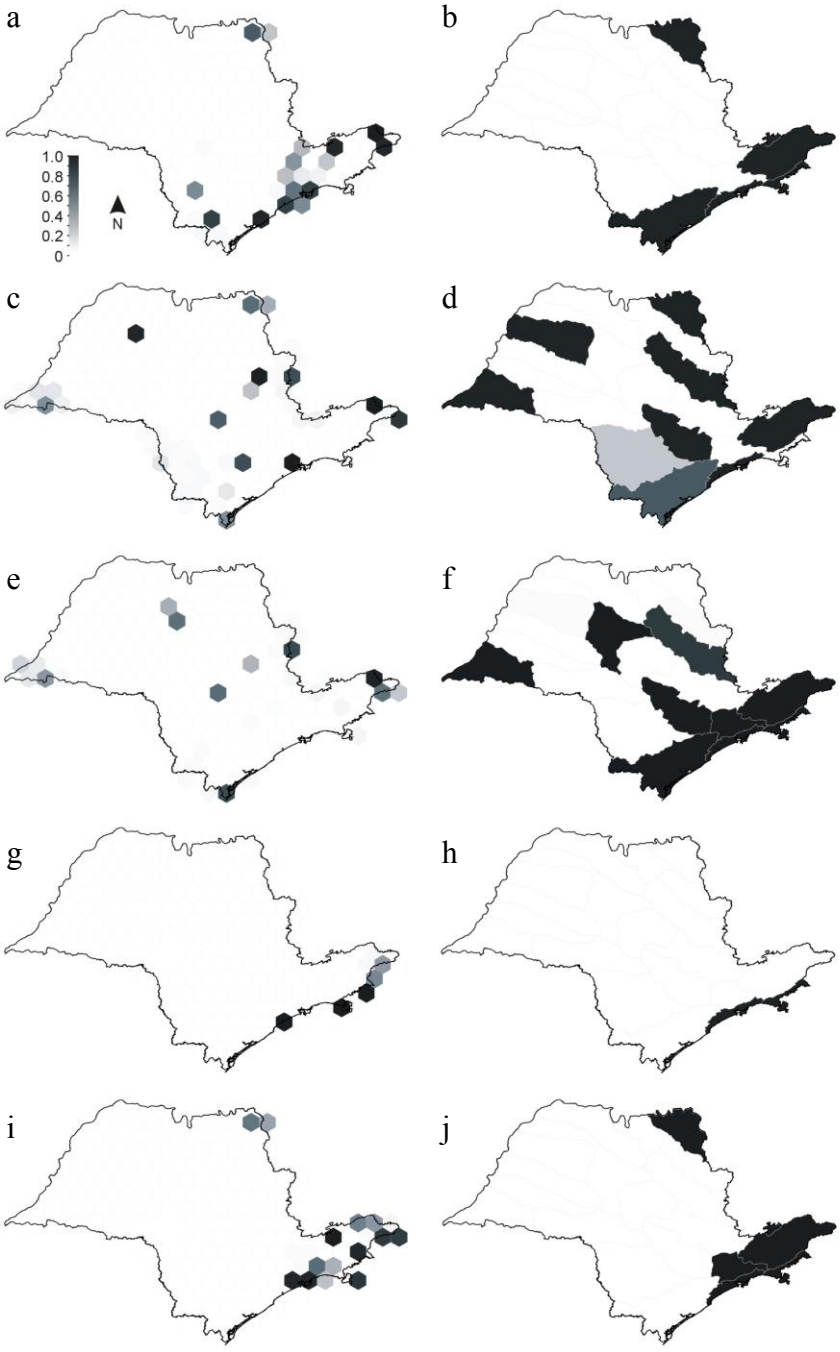

Figure 1.4: Spatial distribution of grid cells or watersheds revealed by their selection frequency in the 100 solutions obtained for frog species breeding in flowing water – FW (a, b); still water/permanent – SP (c, d); still water/temporary – ST (e, f); bromeliad or bamboo – BB (g, h) and breeding terrestrially by direct or indirect development – DI (i, j). Scale represents selection frequency.

After taking into consideration the grid cells already protected in state and national parks, we found that a substantially smaller number of grid cells is required to complement the original network of protected areas and protect all amphibian species. We detected 17 priority grid cells necessary to represent all species at least once (Figure 1.5). Most of the cells protect species with only one (six cells) or two (10 cells) reproductive habitats. We found only one cell representing species with four habitat requirements, thus emphasizing the importance of considering life-history traits in amphibian conservation planning. The highest priority areas for preserving species that breed in flowing and permanent still water were concentrated in the southern regions of the state, while those crucial for species breeding in temporary ponds were concentrated in central and eastern regions (Figure 1.5). The highland regions of Serra da Mantiqueira and Serra da Bocaina are key areas for preserving species with a variety of reproductive habitats, including those breeding in flowing and still water as well as breeding by direct and indirect terrestrial development. Species breeding in bromeliads and bamboos were already well represented in protected areas (Figure 1.5).

Discussion

One of the largest challenges for tropical conservation biology is to develop methods to accurately prioritize conservation efforts. Our results emphasize that linking information about landscape configuration and species life-history can produce more ecologically relevant amphibian conservation strategies, because developmental mode dictates how species respond to given patterns of deforestation (Becker et al., 2007). Integrating life-history and patterns of deforestation can therefore guide the

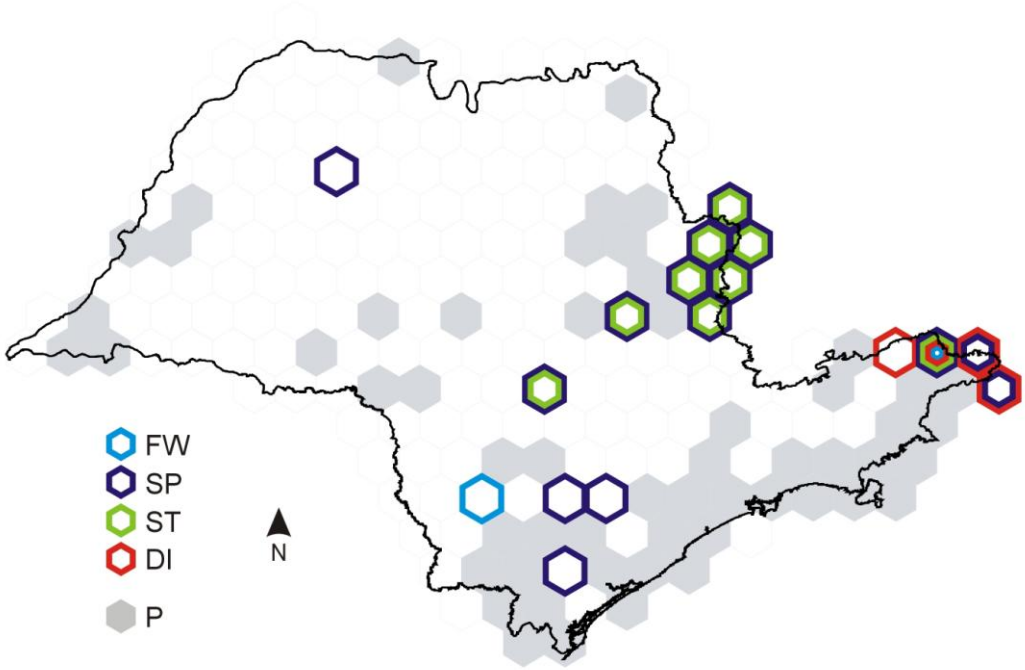

Figure 1.5: Spatial distribution of priority area sets revealed incorporating grid cells with protected areas (IUCN I–IV categories) higher than 300 ha – P (grey). Reproductive habitats as follows: flowing water – FW (light blue), still water/permanent – SP (dark blue), still water/temporary – ST (green), terrestrial by direct or indirect development – DI (red).

location and types of habitats to be restored for most efficient biodiversity conservation.

In the state of São Paulo, we found different patterns of distribution of high-priority areas to protect species requiring distinct reproductive habitats, underscoring the importance of considering species-landscape interactions in prioritization efforts (Loyola et al., 2008a). Our results indicate that different regions will be favoured by distinct restoration plans. Conservation efforts in regions aiming to protect species breeding in flowing and permanent still waters should focus on restoration of riparian buffers, especially in southern coastal regions. These regions are needed to protect species requiring connectivity between aquatic and terrestrial habitats to complete their life cycles. In regions with high habitat split, such as coastal areas of São Paulo (see Figure 1.1), amphibians with aquatic larvae often migrate during the wet season from upland forest fragments to streams and permanent ponds in the disturbed valley (Becker et al., 2010). In the dry season, most species seek shelter in the natural terrestrial habitat, forcing species to move to upland forest fragments and exposing them to the multiple hazards of unnatural migrations (Becker et al., 2010).

In contrast, priority areas aiming to protect species with terrestrial development should focus on restoration of forested habitats. In the Brazilian Atlantic Forest, many species with terrestrial development can avoid crossing disturbed open habitats because they have lower dependence on surface waters for reproduction (Haddad & Prado, 2005). However, this reduced migration along fragmented landscape can result in isolated and patchy populations that will be more prone to the negative effects of inbreeding and bottlenecks (Peakall & Lindenmayer, 2006; Dixo et al., 2009). For

species with direct and indirect terrestrial development, corridors among large forest fragments and safeguarding large forest preserves will be the most effective conservation strategy. In addition, the fact that all species breeding in bromeliads and bamboo stems are represented in forests inside protected parks underscores the need for strict enforcement of laws protecting these northern coastal reserves (see Figure 1.4g-h).

Our results corroborate previous studies showing the importance of explicitly accounting for the existing protected-area networks in defining priorities for future conservation efforts. Once we considered protected areas in our analyses, fewer additional grid cells were required to complement the established reserve network in the state of São Paulo. The hottest 'hotspot' for amphibian conservation in the state includes segments of the Serra da Mantiqueira and Serra da Bocaina mountain ranges in southeastern São Paulo. In this region, we found four priority grid cells for conserving species with a variety of reproductive habitats (see Figure 1.4). Our results show that amphibians of São Paulo would benefit from new protected areas along Serra da Mantiqueira (see appendix), where continuous natural habitat is needed to safeguard endemic species with a variety of habitat requirements.

Not surprisingly, most grid cells and watersheds with high selection frequency cluster in regions with high species richness that also coincide with historical habitat stability (Carnaval & Moritz, 2008; Carnaval et al., 2009), underscoring how unique patterns of diversification in this area also impact prioritization decisions. Prioritization studies at different spatial scales and using alternative biogeographical units of analysis can sometimes produce contrasting outcomes (Larsen & Rahbek, 2003), yet our results

revealed the same patterns and priorities based on grid cells and watersheds. Much of the debate on the biases due to spatial scale has focused on the accuracy of predicting biodiversity patterns (Rahbek & Graves, 2001), whereas less attention has focused on issues of scale in conservation planning (Larsen & Rahbek, 2003). Our results indicate that it should be possible to use larger geographical units, such as watersheds or ecoregions, as a coarse filter for establishing conservation priorities (Loyola et al., 2008b) in other regions of the Atlantic Forest. Because the priority areas obtained using different geographical units were spatially congruent, larger units could be identified first using landscape features that naturally delimit regions of biodiversity, and then scaled-up to higher resolution to produce targeted conservation proposals.

Prioritization analyses should be considered indicative of the potential efficacy of future conservation efforts, rather than prescriptive (Valenzuela-Galvan & Vazquez, 2008). These analyses will be most useful to conservation planners as a means of assessing potential costs in achieving particular conservation goals. The identification of a comprehensive set of natural areas is only the first step toward an in situ biodiversity conservation strategy, which requires a more complex process of policy negotiation and implementation (Loyola et al., 2008a). Final decisions should be based on comparing alternatives and considering the interests of all stakeholders (Pressey et al., 1997). Our scenarios are no substitute for this integrated negotiation process, but they are part of a wide-ranging effort (Soutullo et al., 2008) to strengthen the scientific basis for conservation decisions.

Our study is novel in its attempt to assess conservation needs independently for frogs with different life-history traits. In a previous work (Becker et al., 2007) we

clearly demonstrated that mechanisms of endangerment are different for aquatic and terrestrial breeding amphibians, and we show here that taking those mechanisms into account influences the optimum prioritization scheme and restoration targets (see also Loyola et al., 2008a). After restoration, continued monitoring of the outcome of conservation efforts should help us identify further specific biological characteristics that provide maximum returns for conservation (Margules & Pressey, 2000). Because species vary in their conservation requirements, evidence based conservation decisions are necessary to take into account the multiple characteristics that might be ecologically relevant (Svancara et al., 2005).

The coastal region of São Paulo, where deforestation is more prevalent in riparian zones, harbours not only the majority of irreplaceable areas but also the highest species richness. The majority of cells with high selection frequency in this region protect species with aquatic larvae. This result illustrates why habitat split should be considered in restoration programmes for the Brazilian Atlantic Forest, to curb deforestation that specifically occurs in riparian zones (Figure 1.1). If current legislation protecting riparian vegetation were adequately enforced (Brazilian Forest Code 4771/65), the degree of habitat split for amphibians would be low in the State of São Paulo, even under scenarios of substantial habitat loss. Preventing further habitat split may help safeguard the majority of amphibian species in this diverse region because most (80%) anurans recorded in the state have aquatic larvae. Preservation of riparian forests is an important step for biodiversity conservation more generally, and not only for amphibians. Riparian zones mitigate the effects of deforestation on other organisms such as fishes (Lorion & Kennedy, 2009), birds (Smith et al., 2008) and

invertebrates (Sweeney et al., 2004). Forested riparian zones provide allochthonous organic matter inputs (e.g., large woody debris, leaf litter, terrestrial insects and vertebrates) that serve as food and habitat for stream organisms (Sweeney, 1993), provide shade that moderates water temperature (Abell et al., 2007) and maintain stream channel features (Sweeney et al., 2004). The fact that forested riparian zones also filter sediments, nutrients and pollutants from agricultural runoff (Osborne & Kovacic, 1993) make the preservation and restoration of these habitats a general priority.

Habitat loss figures prominently in global conservation assessments (see Brooks et al., 2006), but additional attention to the spatial configuration of both terrestrial and aquatic habitats will improve the design of reserve networks for amphibian conservation at the regional scale (Crawford & Semlitsch, 2007; Gardner et al., 2007). Legal requirements to maintain forest cover within riparian zones combined with the need to maintain access to sources of clean water present a good opportunity to work with landowners to enhance amphibian conservation strategies in fragmented landscapes. A lack of careful land-use planning in many countries has led to the disproportionate destruction of riparian vegetation, causing a drastic reduction in the availability and quality of water (Sweeney et al., 2004). As a consequence, water is becoming a scarce resource for a wide range of organisms, including humans (Daily, 1997). Our results highlight the importance of curbing further habitat split, because once it occurs, only extensive restoration programmes (Wuethrich, 2007) will reverse its negative impacts. In the Brazilian Atlantic Forest, conserving forested habitats, but also considering connectivity among terrestrial and aquatic habitats will be necessary

to design effective conservation strategies to safeguard its megadiverse and imperiled fauna.

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CHAPTER 2

TROPICAL AMPHIBIAN POPULATIONS EXPERIENCE HIGHER DISEASE RISK IN NATURAL HABITATS

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Abstract

Habitat loss and disease are main drivers of global amphibian declines, yet the interaction between them remains largely unexplored. Here we show that paradoxically, habitat loss is negatively associated with occurrence, prevalence, and infection intensity of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in amphibian populations in the tropics. At a large spatial scale, increased habitat loss predicted lower disease risk in amphibian populations across Costa Rica and eastern Australia, even after jointly considering the effect of potential biotic and abiotic correlates. Lower host-species richness and suboptimal microclimates for *Bd* in disturbed habitats are potential mechanisms underlying this pattern. Furthermore, we found that anthropogenic deforestation practices biased to lowlands and natural vegetation remaining in inaccessible highlands explain increased *Bd* occurrence at higher elevations. At a smaller spatial scale, holding constant elevation, latitude, and macroclimate, we also found a negative relationship between habitat loss, and both *Bd* prevalence and infection intensity in frog populations in two landscapes of the Brazilian Atlantic Forest. Our results indicate that amphibians will be disproportionately affected by emerging diseases in pristine environments, and that, paradoxically, disturbed habitats may act as shelters from disease, but only for the very few species that can tolerate deforestation. Thus, tropical amphibian faunas are threatened both by destruction of natural habitats as well as increased disease in pristine forests. To curb further extinctions and develop effective mitigation and restoration programs we must look to interactions between habitat loss and disease, the two main factors at the root of global amphibian declines.

Introduction

Habitat loss and chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), are two main causes of global amphibian declines (1–4). Habitat loss lowers amphibian species diversity by reducing natural habitats (1) and increasing population isolation (5), inbreeding (6), edge effects (7), and discontinuity between terrestrial and aquatic habitats (8). Disturbance to natural vegetation also changes ecosystem structure, shifting macro (9) and microclimates (10), and altering hydrological cycles (11). Thus, habitat loss may also influence amphibian susceptibility to disease by altering host-community structure, transmission pathways, and pathogen persistence and virulence (12). *Bd* is a water-borne epidermal pathogen with a broad host range among amphibians (13) and has been implicated in population declines and species extinctions worldwide (3, 4, 14–16). *Bd* prevalence and infection intensity are important predictors of disease risk and population die-offs (17) and, to some extent, can be modified by environmental factors. *Bd* prevalence varies with latitude (18), elevation (19), precipitation (18, 20, 21), and temperature (18, 20), presumably reflecting *Bd* optimal growth conditions (22, 23).

The most severe amphibian declines and extinctions have been observed in the Neotropics (15) and Australia (14). In both regions, *Bd* outbreaks occur primarily in pristine forests at high elevation mountainous sites, such as the Talamancas in Central America (15, 24), the Tropical Andes in South America (15), and the Great Dividing Range in eastern Australia (25). In contrast, pathogen prevalence is lower and amphibian populations often remain stable at lowland sites (15, 19, 25). Because *Bd* optimal growth occurs at mild temperatures (17–25 °C) and high humidity (22, 23),

these regional patterns of declines could result from the interaction among elevation, climate, and also habitat loss, all of which are potentially correlated. Temperature is negatively correlated with elevation. Elevation and habitat loss are often negatively correlated because of anthropogenic deforestation practices that are biased to the flatter and more accessible lowlands (26). Finally, tropical habitat loss increases average temperatures and may change precipitation patterns (11). In the case of *Bd*, most empirical studies have focused on the interaction of only two of these three critical components, elevation and climate, eclipsing the potential role of habitat loss and its cascading biotic and abiotic consequences on *Bd* epidemiology at both large and small spatial scales.

Here, we examined the effect of habitat loss on *Bd* infections in tropical amphibian populations at both large and small spatial scales. We hypothesized that habitat loss is negatively associated with *Bd* infections because of lower host-species richness and potentially suboptimal microclimate for *Bd* in disturbed habitats. First, we analyzed published surveys of *Bd* infections of Rain frog populations (*Craugastor fitzingeri*) in Costa Rica (20), and Stony Creek frog populations (*Litoria lesueuri*) in eastern Australia (18). For each sampling site we quantified the degree of habitat loss, measured as the percentage of non-natural vegetation cover within 1-km buffers surrounding sampling locations. We tested the effect of habitat loss on pathogen occurrence, prevalence, and infection intensity using spatial autoregressions and path analysis, and accounted for potential effects of host-species richness, latitude, elevation, and bioclimatic metrics of temperature and precipitation. In a second study, replicated in two landscapes of the Brazilian Atlantic Forest, we compared *Bd*

prevalence and infection intensity in populations of the Golden Lesser treefrog (*Dendropsophus minutus*) among sites with varying levels of habitat loss. At this smaller scale, we controlled for the effects of environmental factors by sampling populations with similar climate, elevation, and latitude, and used spatial autoregressions to test the relationship of habitat loss with *Bd* prevalence and infection intensity.

Results

Habitat Loss Predicts Bd *Infections in Costa Rica and Australia.*

Increased habitat loss predicted lower *Bd* occurrence in amphibian populations in Costa Rica ($\beta_{AUTOLOG} = -0.022$, $t = -1.953$, $P = 0.051$). Our initial stepwise screening for explanatory variables selected habitat loss, amphibian species richness, elevation, temperature annual range, and precipitation of the driest quarter of the year as variables with high scores for explaining *Bd* occurrence. When jointly considering these effects in a model selection approach, including all possible models, habitat loss remained a strong predictor of pathogen occurrence (Table 2.1 and Table S2.1). In the best model, we found a negative effect of habitat loss and a positive effect of species richness on *Bd* occurrence.

Anthropogenic deforestation is nonrandomly distributed throughout Costa Rica because habitats in flatter lowlands are disproportionately disturbed and, thus, natural vegetation persists at higher elevations (Figure 2.1a-b). This nonrandom pattern potentially confounds the effects of elevation and habitat loss on pathogen occurrence. We considered the influence of elevation on habitat loss in a binary response path

Table 2.1: Autologistic and conditional autoregressive models testing simultaneously the effects of habitat loss, amphibian species richness, and environmental factors on *Bd* occurrence in amphibian populations in Costa Rica, and on *Bd* prevalence and infection intensity in amphibian populations in Australia.

Term	$\beta_{AUTOLOG}$	StdCoeff.	SE	*t*	VIF	*P*
OCCURRENCE						
Constant	*-6.311*	*0*	*2.033*	*-3.104*	*.*	*0.002*
Spatial Auto-covariate Term - yW	*3.138*	*0.879*	*3.075*	*1.020*	*.*	*0.308*
***1* Habitat Loss**	**-0.030**	**-3.769**	**0.012**	**-2.430**	**1.020**	**0.015**
***2* Amphibian Species Richness**	**0.098**	**4.224**	**0.042**	**2.318**	**1.020**	**0.020**

Term	β_{CAR}	StdCoeff.	SE	*t*	VIF	*P*
PREVALENCE						
Constant	*-15.98*	*0*	*33.36*	*-0.479*	*.*	*0.636*
***1* Habitat Loss**	**-0.304**	**-0.420**	**0.113**	**-2.695**	**1.279**	**0.012**
***2* Amphibian Species Richness**	**1.414**	**0.466**	**0.658**	**2.148**	**2.059**	**0.042**
***3* Latitude & Max Temp Warmest Month PC1[1]**	**-36.959**	**-2.179**	**11.699**	**-3.159**	**2.470**	**0.004**
*2 * 3*	0.796	1.439	0.390	2.039	1.355	0.052

Term	β_{CAR}	StdCoeff.	SE	*t*	VIF	*P*
INFECTION INTENSITY						
Constant	*0.555*	*0*	*1.439*	*0.386*	*.*	*0.703*
***1* Habitat Loss**	**-0.017**	**-0.416**	**0.006**	**-2.626**	**1.025**	**0.014**
***2* Precipitation of the Driest Month**	**0.043**	**0.544**	**0.011**	**4.001**	**1.025**	**<0.001**

Whole model tests: Occurrence (χ^2 = 14.888, N = 125, *P* = 0.002); Prevalence (*F* = 8.700, N = 31, r^2_{OLS} = 0.581, *Predictor+Space* r^2 = 0.632, *P* < 0.001); Infection Intensity (*F* = 10.491, N = 31, r^2_{OLS} = 0.458, *Predictor+Space* r^2 = 0.582, *P* < 0.001). Significant variables in the model are highlighted in bold. VIF stands for variance inflation factor and denotes colinearity in the model if higher than 10. Final models chosen based on AIC. [1] PC1 consolidating latitude and maximum temperature of the warmest month accounted for 96.00 % of the variation in the original variables.

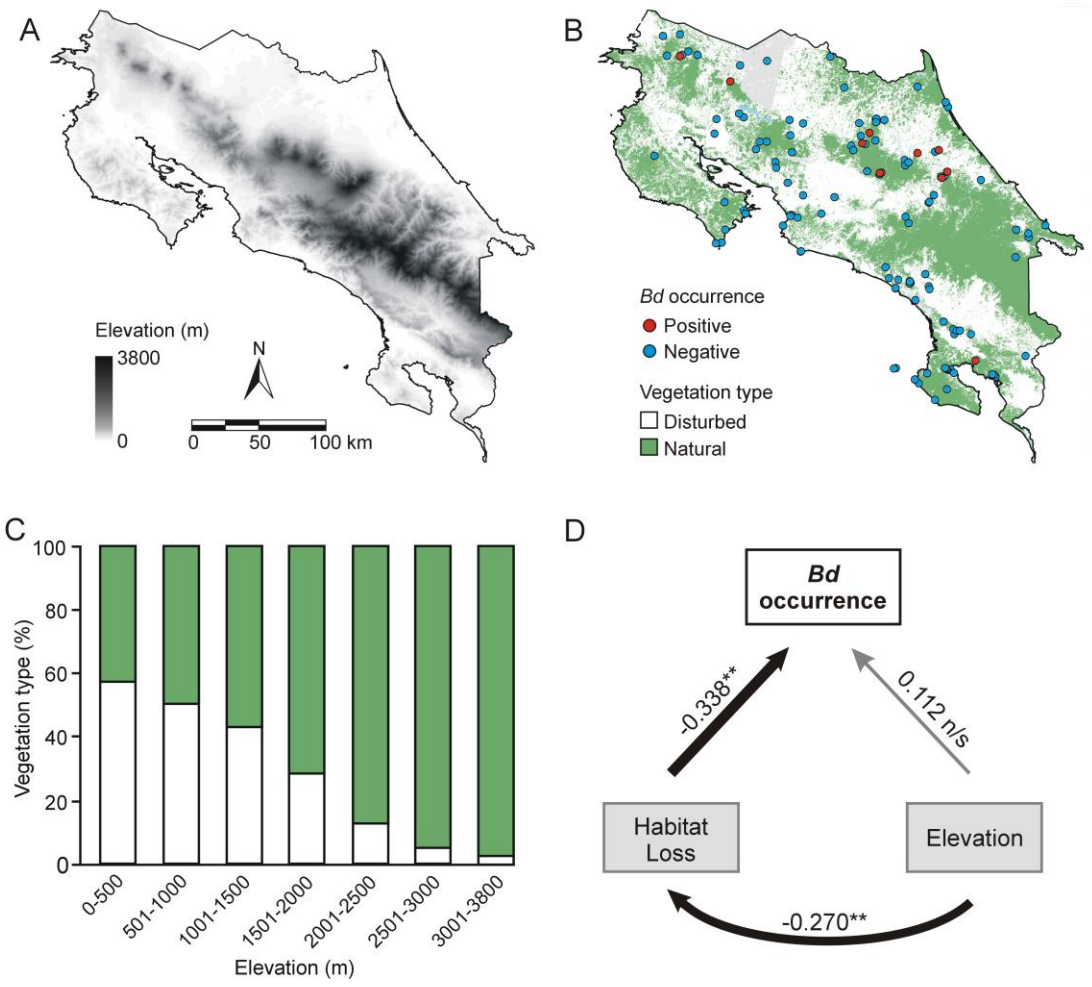

Figure 2.1: (a) Elevation range and (b) spatial distribution of disturbed and natural vegetation throughout Costa Rica. Sampling sites are *Bd* positive (red circles) and negative (blue circles); vegetation types are disturbed (white) and natural (green); other land-cover classes are freshwater (light blue) and unclassified cloud cover (gray). (c) Percentage of disturbed (white) and natural vegetation (green) across the altitudinal gradient. (d) Path Analysis model showing the relative strength of habitat loss and elevation on *Bd* occurrence among amphibian populations. Numbers are standardized path coefficients (**P < 0.01). The thickness of the arrows represents the relative strength of the relationship.

analysis and showed that the effect of elevation on *Bd* occurrence is indirect through habitat loss (whole-model test: $\chi^2_{[3]}$=22.545, P <0.001) (Figure 2.1d).

Increased habitat loss also predicted lower *Bd* prevalence in amphibian populations across eastern Australia ($\beta_{CAR} = -0.346$, $t = -2.579$, $P = 0.015$), in this case, without the confounding effects of elevation across sampling locations (18). The initial screening detected habitat loss, amphibian species richness, latitude, maximum temperature of the warmest month, and precipitation of the warmest quarter of the year as potential explanatory variables for *Bd* dynamics. Because latitude and maximum temperature of the warmest month were highly correlated ($r = -0.920$, $P < 0.001$), we included them in the model selection as a PC variable. When jointly considering all effects in model selection, habitat loss remained a strong negative predictor of *Bd* prevalence, amphibian species richness a positive predictor, latitude a positive predictor, and maximum temperature during the warmest month a negative predictor (Table 2.1 and Table S2.1). Habitat loss alone was not a significant predictor of *Bd* infection intensity ($\beta_{CAR} = -0.012$, $t = -1.596$, $P = 0.122$); however, when considered jointly with other variables selected in the initial screening, habitat loss became a strong factor, explaining infection intensity together with precipitation of the driest month (Table 2.1 and Table S2.1). The inclusion of spatial autocorrelation in our analyses improved model fit for all reported models (Table 2.1).

Habitat Loss Predicts Bd *Infections at Small Spatial Scale.*

Habitat loss predicted lower *Bd* prevalence in amphibian populations in both Brazilian Atlantic Forest landscapes. As expected, none of the 19 bioclimatic variables

explained *Bd* prevalence and infection intensity at this smaller spatial scale. We showed that habitat loss alone is a key factor predicting *Bd* prevalence (β_{CAR}= −0.556, t = −5.074, P = 0.001) (Figure 2.2a) and infection intensity (β_{CAR} = −0.027, t = −5.564, P < 0.001) (Figure 2.2b) in Araucaria Moist Forest, and although not statistically significant, the same trends were found for the Serra do Mar Coastal Forest [prevalence: β_{CAR} = −0.371, t = −1.683, P = 0.136 (Figure 2.2c); infection intensity: β_{CAR} = −0.017, t = −1.520, P = 0.172 (Figure 2.2d)]. Because host population density can differ across disturbance gradients, we examined the effect of amphibian capture rate (as a proxy for population density) on both *Bd* prevalence and infection intensity and found no significant relationships [Serra do Mar Coastal Forest (β_{CAR} = −2.402, t = −1.306, P = 0.233; β_{CAR} = −0.168, t = −1.966, P = 0.090), Araucaria Moist Forest (β_{CAR} = −4.695, t = −1.475, P = 0.184; β_{CAR} = −0.200, t = −1.324, P = 0.227)].

Discussion

We showed that habitat loss, a main cause of species extinctions worldwide (27), is negatively associated with *Bd* occurrence, prevalence, and infection intensity in tropical amphibian populations. This effect was evident even when jointly considering several known environmental correlates of pathogen growth and persistence (18–21, 24). We corroborated these results at a smaller geographic scale, where habitat loss predicted lower pathogen prevalence and infection intensity in populations with similar macroclimate, topography, and latitude. Thus, localized differences in natural vegetation cover may explain why neighboring populations often have highly contrasting *Bd* dynamics. Our path analysis showed clearly that elevation had indirect

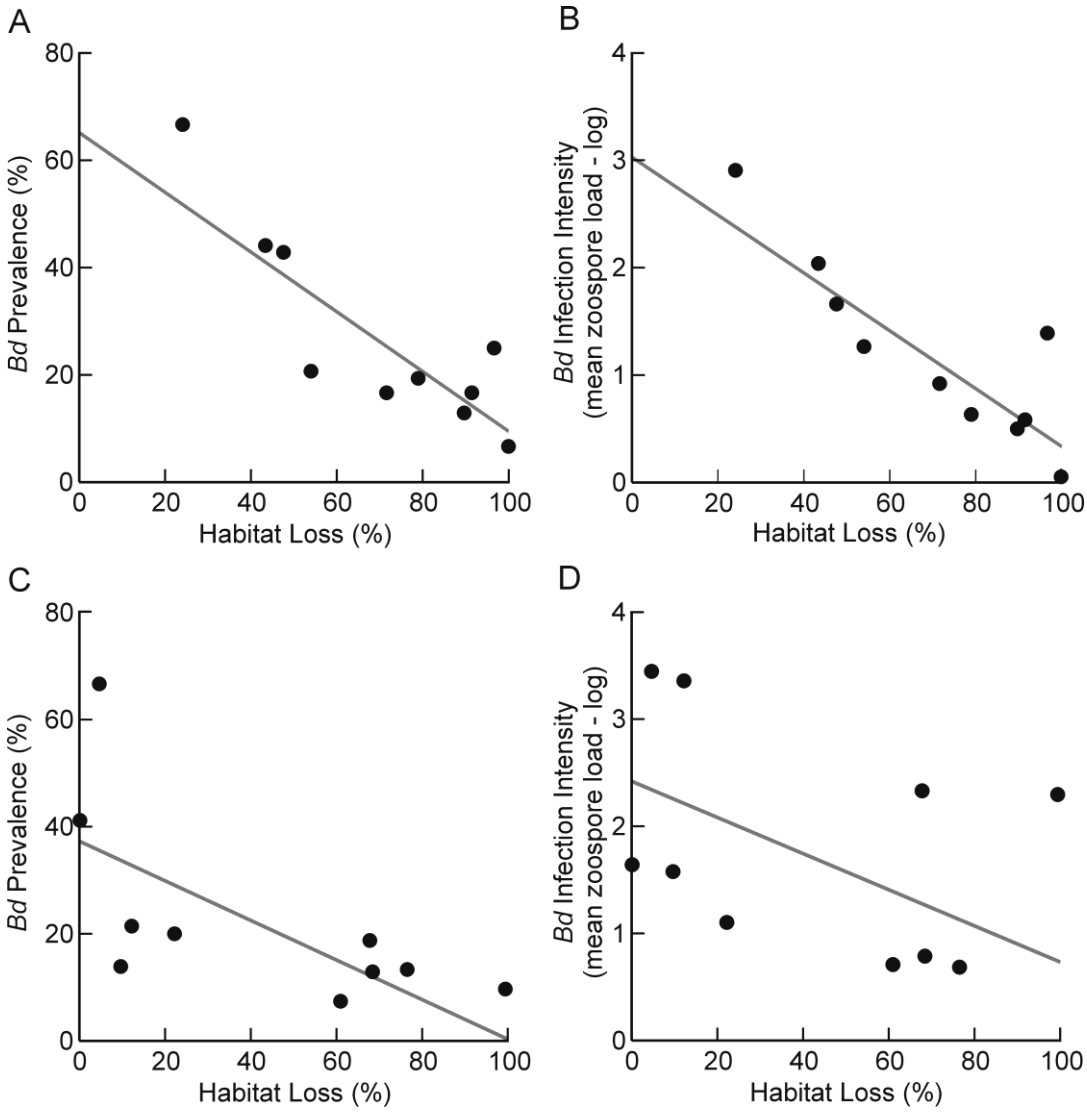

Figure 2.2: Effect of habitat loss on *Bd* prevalence and infection intensity across populations of *D. minutus* in two Brazilian Atlantic Forest landscapes: (a and b) Araucaria Moist Forest, southern Brazil and (c and d) Serra do Mar Coastal Forest, southeastern Brazil.

effects on *Bd* occurrence via habitat loss. Therefore, the role of habitat loss as a determinant of amphibian disease threat has likely been underestimated because of nonrandom deforestation practices relative to topography.

Our analyses demonstrate a negative relationship between habitat loss and disease risk on three continents and at two spatial scales, despite many studies showing the opposite pattern. Habitat loss disrupts natural ecosystems and often increases the risk of human and wildlife diseases (12, 28). Tropical deforestation coincides with a rise of malaria in Africa, Asia, and Latin America, independent of human population density (12, 29). Habitat change was positively associated with the emergence of bat-borne Nipah virus in Malaysia (30), cryptosporidiosis in Europe and North America, and food-borne illnesses globally (31). Bighorn sheep populations in the San Andres Mountains, New Mexico, suffered demographic declines because of severe habitat loss and fragmentation and were subsequently stricken by an epidemic of psoroptic scabies (28). A possible mechanism for this general pattern is that habitat loss limits individual movement and dispersal, which in turn increases disease risk because of elevated stress and contact rates among individuals in crowded patchy populations (32). Population-based models of disease dynamics indicate a potential trade-off in that habitat fragmentation can limit pathogen transmission between small and isolated populations, but it also increases genetic erosion of resistant alleles and reduces the probability of rescue events, increasing the demographic consequences of disease epidemics (33).

In the case of *Bd*, there is mounting evidence that humans have been playing a key role in spreading the pathogen (3, 34, 35), with infections often higher in areas of higher human footprint (36). Surveys in both temperate and tropical regions revealed

that *Bd* detectability increased with human population density, and in the vicinity of port cities and the highways connecting them (37). Our results, however, show that anthropogenic habitat loss, specifically deforestation, can lower *Bd* infections in amphibians. This pattern was also observed in *Litoria wilcoxii* populations of Eastern Australia, where forested habitats harbored amphibians with higher pathogen prevalence then neighboring agricultural lands (38). Other forms of habitat change may also decrease *Bd* fitness: small populations of *Litoria aurea* persisted in heavy industrial and mining areas after a severe outbreak of chytridiomycosis, indicating that *Bd* may be sensitive to environmental contaminants (39). Likewise, in a pristine region of Eastern Australia where *Bd* was previously documented, forest-associated amphibians suffered local extinctions, but species richness remained high in adjacent urban areas (40). Combined, these examples and our results suggest that although humans may assist pathogen dispersal, anthropogenic habitat changes may also limit *Bd* persistence.

We propose two potential mechanisms for elevated disease risk in pristine environments: (i) lower *Bd* infection risk at disturbed habitats because of lower host-species richness, and (ii) suboptimal microclimatic conditions for *Bd* in disturbed habitats. *Bd* is one of the most host-generalist pathogens ever found, with over 350 amphibian host species documented to date (3, 13). Therefore, higher biodiversity and community complexity might amplify *Bd* infections, because greater diversity of host species can enhance pathogen transmission due to higher availability of susceptible hosts throughout the year. We found a positive relationship between amphibian species richness and *Bd* infections in Costa Rica and Australia, where the negative effects of

habitat loss on amphibian species richness are well documented (41, 42). Thus, habitat loss may indirectly reduce *Bd* infections by lowering species richness in amphibian communities. Although we do not have data on species richness for our small-scale study, deforestation along riparian zones is shown to decrease amphibian species richness in the Brazilian Atlantic Forest (2), thus the same potential mechanism may apply for our smaller scale landscape studies. Different disease outcomes across natural populations may result from density-dependent host-pathogen dynamics (43); however, we found no significant effect of capture rate (a proxy for host population density) on *Bd* prevalence and infection intensity. Clearly, the relationship between biodiversity and disease dynamics is complex (44) and deserves further investigation.

A second potential mechanism for decreased *Bd* infections in disturbed habitats is suboptimal microclimatic conditions for the pathogen arising from habitat loss. Habitat loss changes microclimate (10), potentially shifting temperature and humidity to levels that limit *Bd* growth and persistence (22). This mechanism accounted for the rise of Sudden Oak Death in northern California, where the pathogen thrived in areas of natural vegetation cover with milder temperatures, higher understory humidity, and reduced temperature oscillations, resulting in higher inoculum load in infected trees in closed forest (45). Multiple studies have investigated the role of regional climate in *Bd*-induced amphibian declines (24, 46, 47). However, few studies to date have focused on how microclimate mediates host–pathogen interactions (48, 49), and more broadly, how large-scale climate relates to microclimates experienced by individual frogs. A recent study found that ponds with fewer surrounding trees reached higher temperatures, significantly reducing *Bd* infections in salamanders (49). Given the

known temperature-dependence of host–pathogen interactions, another hypothesis is that temperature variability, rather than average temperature, is an important predictor of disease risk (47). Increased temperature variability reduces host-immunological response against pathogens (50). If habitat loss increases microclimatic variability, resulting in immunocompromised hosts, we would expect increased disease risk, a pattern opposite to the one we found. Conversely, if habitat loss increases temperature variability with extremes beyond the *Bd* optimal temperature range, this will result in reduced *Bd* infections. Therefore, the outcome for disease risk will depend on the relative effects of microclimatic conditions on the host and the pathogen.

We found that habitat loss reduced *Bd* prevalence and infection intensity in species that are wide-ranging habitat generalists and persist across disturbance gradients. These results raise the obvious question of how habitat loss and disease will impact *Bd*-susceptible amphibians with narrow ranges and habitat specialization. Most tropical amphibians are in fact specialists, with on average smaller geographic ranges, more patchy distributions, and more restricted microclimatic tolerances (51), limiting their ability to escape infections in habitats less suitable for *Bd*. Thus, habitat specialists, such as species in the genus *Atelopus*, potentially suffer negative effects because of both habitat loss and higher *Bd* exposure (15). In fact, *Bd* homogenizes tropical amphibian faunas by targeting endemic species (52), thus the interaction between habitat loss and disease may further increase the risk of extinction among habitat specialists.

In this age of biodiversity crisis, it is paradoxical that amphibians can be disproportionately affected by emerging diseases in pristine tropical forests, and that

disturbed habitats might act as refuges from disease for the few species that can tolerate deforestation. Our findings support the hypothesis that habitat loss is a key factor in *Bd* epidemiology in the tropics, and that associated changes in host community structure and microclimate shifts may be the mechanisms behind this pattern. Therefore, epidemiological studies of amphibian declines should incorporate host population and community attributes as well as microclimate data to elucidate mechanisms that control disease and thus enhance conservation of wild populations. If deforestation is consistently biased to lower elevations in regions experiencing amphibian declines, our findings may have even greater implications for predicting *Bd* spread, especially in the context of environmental determinants and infection patterns at smaller spatial scales, those ecologically relevant at the individual level. The emerging field of spatial epidemiology, bridging landscape and disease ecology (53), provides a more complete picture of amphibian population declines and extinctions. Most tropical amphibians are threatened both by destruction of natural habitats and increased disease in pristine areas. Thus, our results indicate that to curb further extinctions and develop effective mitigation and restoration programs, we must look to interactions between habitat loss and disease: the two main factors at the root of the global decline of amphibians.

Materials and Methods

Large Spatial Scale Data

We used published datasets of *Bd* occurrence among populations of the Common Rain frog (*Craugastor fitzingeri*, Craugastoridae) in Costa Rica (20), and *Bd*

prevalence and infection intensity for populations of the Stony Creek frog (*Litoria lesueuri* complex, Hylidae) across eastern Australia (18). *C. fitzingeri* breeds terrestrially and *L. lesueuri* breeds in streams; both species are habitat generalists and populations persist with *Bd* (18, 20). Sampling sites for *C. fitzingeri* were distributed throughout Costa Rica (125 sites; 349 sampled frogs), ranging from sea level to elevations up to 2,110 m. Frogs were sampled between 1993 and 2008, after the arrival of *Bd* in that country (15). *Bd* infection in frogs was assessed using histological screening (20). Because of uneven sampling efforts across populations, we considered only occurrence (presence or absence) of *Bd* at each sampling site. Sampling sites for *L. lesueuri* ranged from northern Queensland to southern New South Wales (31 sites; 863 sampled frogs), along the eastern slope of the Great Dividing Range. To control for elevation and seasonality all sampling occurred at lowland sites (mean elevation 84.51±49.7 SD), and within a 42-d period during the spring of 2005. Australian frogs were swabbed in the field and *Bd*-screened using quantitative PCR (qPCR) (18). *Bd* infection was assessed at the individual level. Prevalence was estimated as the percentage of infected individuals per population, and infection intensity as the mean number of "zoospore DNA equivalents" for all individuals at each population (18).

We obtained land-cover information from Fondo Nacional de Financiamento Forestal for Costa Rica [30-m resolution; coverage period 1995 2005 (54)], and from the Bureau of Rural Sciences for Australia [100-m resolution; coverage period 1995–2000 (55)]. We acquired elevation data (90-m resolution) from the Consultative Group on International Agricultural Research Consortium for Spatial Information (56). Nineteen bioclimatic variables for both studies were extracted using

Worldclim/Bioclim layers (1000-m resolution), available at http://www.worldclim.org/bioclim (57). These metrics of temperature and precipitation are averaged from 50-y records (1950–2000) from a dense network of climatic stations throughout the world (e.g., precipitation records from 47,554 locations, temperature from 24,542 locations). We did not include temperature variability or any variable accounting for climate change in our analyses because samples for *Bd* diagnosis were collected over short time periods, precluding longer-term temporal analyses of *Bd* dynamics. We obtained amphibian species richness at each sampling locations in Costa Rica and Australia by overlaying GIS shapefiles of species historical geographic ranges from the Global Amphibian Assessment (16).

We created habitat loss layers for Costa Rica and Australia as the percentage of nonnatural vegetation cover ranging from 0% to 100% at 1-km resolution. Land-cover types considered as nonnatural were urbanization, pasture, agriculture, and exotic crops. Natural vegetation types were primarily forest habitats. All variables were measured at 1-km pixel to maintain a consistent scale across the analyses.

Small Spatial Scale Data

We sampled 20 populations of the Golden Lesser treefrog (*Dendropsophus minutus,* Hylidae) in two landscapes of the Brazilian Atlantic Forest in southern and southeastern Brazil [10 populations in Araucaria Moist Forest, State of Rio Grande do Sul (29° 24′ S, 50° 24′ W), and 10 populations in Serra do Mar Coastal Forest, State of São Paulo (23° 20′ S, 45° 12′ W)]. Maximum distance between sampling locations was

21.33 km for Araucaria Moist Forest and 18.88 km for Serra do Mar Coastal Forest. Our focal species is a habitat generalist that breeds in ponds during the austral summer.

We swabbed frogs in the field (537 sampled frogs; average of 26.85 frogs per site) with sterile swabs for later *Bd* quantification in the laboratory. We screened samples for *Bd* in singlicate using Taqman qPCR (58), extending the range of the standards to 1,000 zoospore DNA equivalents to determine the presence of *Bd* and infection intensity. For calculations of *Bd* prevalence, we categorized individuals as *Bd*-positive when zoospore equivalents were ≥ 1. We defined infection intensity as the mean number of "zoospore equivalents" per population. We calculated amphibian capture rate for each population based on individuals sampled per person-hour. We assessed natural vegetation cover for each sampling site based on high-resolution satellite images from 2010 (SPOT, 2-m resolution). In each landscape, the selected study sites represented a gradient of natural vegetation cover. We calculated habitat loss as the percentage of non-natural vegetation cover within a diameter of 600 m and 1 km surrounding each sampling site using ArcGIS 9.3.1 (59). To avoid effects of elevation and macroclimate we chose landscapes with low climatic and topographic variability (mean elevation of sampling sites for Araucaria Moist Forest 918.0 ± 10.2 m SD; for Serra do Mar Coastal Forest 929.9 ± 71.4 m SD). We extracted the same 19 bioclimatic variables (57) for each sampling site at 1-km scale. To avoid biases because of seasonality, we sampled continuously over 30 d in Serra do Mar Coastal Forest, and 41 d in Araucaria Moist Forest in 2009/2010.

Statistical Analyses

To assess the relationship between habitat loss and *Bd* occurrence in amphibian populations among sampling sites in Costa Rica while accounting for spatial autocorrelation, we performed autologistic regressions (AUTOLOG). To investigate the relationship between habitat loss and *Bd* prevalence, and between habitat loss and infection intensity among sampling sites in Australia, we used conditional autoregressions (CAR). After this first univariate assessment, we used stepwise regressions (exclusion cutoff $P < 0.10$; inclusion cutoff $P < 0.20$) to screen for biotic and abiotic factors that potentially predict disease threat to be included in model selection procedures. For each analysis, we screened a total of 23 explanatory variables, including habitat loss, amphibian species richness, latitude, elevation, and the 19 bioclimatic temperature and precipitation metrics. Once important variables were identified for each dataset, we used principal components analysis to consolidate cross-correlated variables, and used the scores of the first PC axis as variables in the subsequent model selection procedure. We used AUTOLOG and CAR model selections, including selected explanatory variables and *Bd* (occurrence, prevalence, or infection intensity) as a response variable. We tested all possible models including interactions. Competing models were ranked based on Akaike Information Criterion (AIC). We reported the best fit model for each run. We assessed multicolinearity in each of the final models using a variance inflation factor.

We used binary response path analysis to statistically model causal relationships among elevation, habitat loss, and *Bd* occurrence in Costa Rica, providing information about the relative strength of the different paths. In the model, elevation

influences habitat loss, and these two variables are allowed to influence *Bd* occurrence independently.

For the small-scale data, we used the same stepwise screening method to confirm that none of the 19 bioclimatic variables explain *Bd* prevalence and infection intensity in both landscapes when accounting for habitat loss (measured at 1-km scale). To quantify the effect of habitat loss on *Bd* prevalence and infection intensity for populations in the two landscapes of the Brazilian Atlantic Forest, we used single CARs. We log-transformed zoospore data (infection intensity) for the analyses. Because climatic variables were not included, final models for both landscapes included habitat loss measured within 600-m diameter buffers surrounding sampling locations. We also ran single CARs to quantify the effect of capture rate on both *Bd* prevalence and infection intensity. Capture rate was used as proxy for population density of *D. minutus* in our analyses of pathogen dynamics. In all analyses at both scales, sampling site was used as the replicate in statistical tests of *Bd* dynamics. We ran analyses using Spatial Analysis in Macroecology v4.0 (60) and Mplus v6.0 (61).

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CHAPTER 3

DISEASE RISK IN TEMPERATE AMPHIBIAN POPULATIONS IS HIGHER AT CLOSED-CANOPY SITES

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Abstract

Habitat loss and chytridiomycosis (a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* - *Bd*) are major drivers of amphibian declines worldwide. Habitat loss regulates host-pathogen interactions by altering biotic and abiotic factors directly linked to both host and pathogen fitness. Therefore, studies investigating the links between natural vegetation and chytridiomycosis require integrative approaches to control for the multitude of possible interactions of biological and environmental variables in spatial epidemiology. In this study, we quantified *Bd* infection dynamics across a gradient of natural vegetation and microclimates, looking for causal associations between vegetation cover, multiple microclimatic variables, and pathogen prevalence and infection intensity. To minimize the effects of host diversity in our analyses, we sampled amphibian populations in the Adirondack Mountains of New York State, a region with relatively high single-host dominance. We sampled permanent ponds for anurans, focusing on populations of the habitat generalist frog *Lithobates clamitans*, and recorded various biotic and abiotic factors that potentially affect host-pathogen interactions: natural vegetation, canopy density, water temperature, and host population and community attributes. We screened for important explanatory variables of *Bd* infections and used path analyses to statistically test for the strength of cascading effects linking vegetation cover, microclimate, and *Bd* parameters. We found that canopy density, natural vegetation, and daily average water temperature were the best predictors of *Bd*. High canopy density resulted in lower water temperature, which in turn predicted higher *Bd* prevalence and infection intensity. Our results confirm that microclimatic shifts arising from changes in natural

vegetation play an important role in *Bd* spatial epidemiology, with areas of closed canopy favoring *Bd*. Given increasing rates of anthropogenic habitat modification and the resulting declines in temperate and tropical frogs, understanding how vegetation cover and disease interact is critical for predicting *Bd* spread and developing appropriate management tools for wild populations.

Introduction

Anthropogenically driven habitat change has important implications for host-pathogen interactions, because even slight changes in environmental conditions can modify numerous biotic and abiotic factors that influence these interactions [1–5]. Habitat modification can alter host-pathogen dynamics by regulating host species richness [1], [6], population size, isolation [7], and inbreeding [8], or by shifting macro [9] and microclimates [10] to conditions detrimental or favorable to hosts or pathogens [11–13]. Therefore, studies investigating the links between habitat change and disease require integrative approaches to control for the multitude of possible interactions in spatial epidemiological research [14].

Shifts in microclimate and changes in host community structure across gradients of habitat alteration play important roles in amphibian epidemiology [6], [15]. The frog killing fungus *Batrachochytrium dendrobatidis* (*Bd*), for instance, is more prevalent and occurs at higher infection intensities in pristine tropical forests compared to disturbed habitats [6]. Typically, shade, humidity, and host diversity are higher in natural forests, whereas temperature and host community evenness are often highest in disturbed areas [10], [16], [17]. This pattern holds both for tropical and

temperate forests. However, decreases in host diversity and local species turnover along gradients of habitat alteration are often less pronounced in temperate zones [18], [19], despite similar changes in microclimate that result from removal of natural vegetation. Thus, temperate amphibian populations that persist in a mosaic of altered landscapes provide an opportunity to investigate the effects of microclimate on amphibian host pathogen interactions in the absence of the strong confounding effects of host diversity on disease dynamics. Even though multiple studies have modeled the role of regional and large scale climate in *Bd*-induced amphibian declines [12], [20], [21], we have not yet fully characterized how habitat change affects local microclimate, which may in turn control pathogen infections [22], [23].

From the host's perspective, immune responses usually decrease as a result of the multiple effects of habitat alteration [24], [25]. Microclimatic changes caused by deforestation can shift thermal physiology and hydric conditions beyond tolerance limits of forest associated amphibians [26], [27]. Because amphibians rely on thermoregulation to maintain homeostasis, changes in temperature and humidity along gradients of natural vegetation can affect their immune responses to pathogens [27], [28]. In addition to temperature variability, exposure to environmental contaminants in disturbed habitats hinders essential components of the host immune system [29–31]. Habitat change also increases stress hormone production, therefore decreasing host immune capacity [24] and increasing susceptibility to disease in nonnatural environments [25], [29]. From the pathogen's perspective, deforestation can shift air and water temperatures to levels that exceed the upper threshold (25°C) of the optimal microclimatic envelope for *Bd*, thus limiting pathogen growth and persistence [32],

[33], [22]. Removal of canopy cover often reduces complexity of aquatic vegetation and of leaf-litter substrates, which might contribute to lower *Bd* persistence in these environments [23]. Therefore, disease risk in amphibian populations will depend on the severity of the environmental change imposed by land-use practices and by the degree to which both hosts and the pathogen respond to the resulting microclimatic changes.

Here, we examined the infection dynamics of *Bd* (prevalence and infection intensity) in populations of the common Green Frog (*Lithobates clamitans*) across a gradient of natural vegetation and microclimate. We sampled frogs in the southern Adirondack Park, New York State, a region with relatively low amphibian diversity and high dominance of this habitat generalist host [34]. Our main goals were to (i) test the hypothesis that natural vegetation surrounding aquatic breeding sites in temperate forests is a significant predictor of *Bd* in amphibians and (ii) test for causal associations linking vegetation cover and microclimate with both *Bd* prevalence and infection intensity. Combined, these goals may elucidate whether vegetation and microclimate modulate disease risk in temperate amphibian populations affected by anthropogenic habitat change.

Methods

Study System

We sampled anuran populations in the Adirondack Park of the State of New York (43°27’ N, 74°67’ W). This region is heavily forested with elements representative of temperate and boreal forests [35], but includes areas with moderate urbanization and agriculture. The fungal pathogen *Bd* is enzootic and widespread in the

northeastern U.S. [36], including the Adirondacks. We sampled ten permanent ponds within a period of 15 days to avoid seasonal effects on host behavior and pathogen dynamics. We restricted our sampling to the period of June18th–July 2nd 2011, when environmental temperatures are suitable for *Bd* growth in this region [36]. We recorded all anurans present in our sampling ponds, but focused on the common Green Frog (*Lithobates clamitans*, Ranidae), the locally dominant amphibian host species. Green Frogs breed in permanent ponds during the boreal spring and summer, and typically spend most of the time at the shallow banks of water bodies [37]. They can tolerate a variety of habitats ranging from closed-canopy to open grassland ponds [37], [38]. We conducted diurnal and nocturnal visual encounter surveys around each of our study ponds with a consistent sampling effort of 4.7 ± 0.75 SD hours.person.pond^{-1}. We recorded body weight (g) for each captured individual and screened post-metamorphic frogs with sterile swabs to quantify *Bd* prevalence and infection intensity (average of 16.6 *L. clamitans* per site). We tested samples for *Bd* in singlicate using Taqman qPCR [39], [40]; with standards of 0.1, 1, 10, 100, and 1000 zoospore genomic equivalents (g.e.) to determine the presence and infection intensity of *Bd* in each sample. This protocol maximizes amplification efficiencies by diluting extracts to reduce inhibition in environmental samples. For calculations of *Bd* prevalence, we categorized individuals as *Bd*-positive when their qPCR showed an infection load of greater than or equal to one g.e. [39], [40]. We defined *Bd* prevalence as the percentage of infected individuals and *Bd* infection intensity as average number of g.e. per population.

Biological and Environmental Predictors of Bd

We recorded average host body weight and capture rate (i.e., captured frogs.person.hour^{-1}) as a proxy for host population density. We specifically chose this study system because of its relatively low species diversity and high single host species dominance. These community attributes allow us to test hypothesis about the roles of microclimate in disease dynamics without the potential effects of complex host community structure. Nonetheless, we recorded host community diversity (Simpson's D [41]) and overall community biomass (i.e., the sum of weights for all captured individuals) for each of our sampling ponds. We assessed natural vegetation cover for each of our 10 sampling sites based on high-resolution orthophotos from 2008–2009 (15 and 30 cm resolution; [42]). For each sampling site, we measured the percentage of natural vegetation cover in a radius of 30 m from the edge of the pond. We considered urban, pasture, agriculture, silviculture, and recreational land (e.g., golf-courses) as nonnatural land-cover types. The selected study sites represented a gradient of cover quality ranging from 2 to 95 percent natural vegetation. We chose sites with low topographic variability (mean elevation of sampling sites 471.3 ± 120.79 m SD) to minimize the effects of elevation and macroclimate on host-pathogen dynamics [43], [44]. Using a canopy densiometer in the field, we measured fine scale canopy density (% canopy cover) at 10 m intervals along the water line [45] and averaged these records for each pond. Although we expect that the GIS-based measurements of natural vegetation will be positively correlated with canopy density measured in the field, natural vegetation can vary considerably in height and leaf coverage. Thus, canopy

density is a better index of vegetation structure, shade, and understory microclimate. We recorded surface water temperatures (i.e., daily average temp., average daily maximum temp., and average daily minimum temp.) for each sampling pond using waterproof data loggers (Hobo UA-002-64; 0.1°C resolution). We placed one data logger in each pond at 10 cm depth at the shallow margin where amphibian captures were concentrated. We used 30 min interval temperature records taken simultaneously at all ponds for a period of 15 days following completion of sampling at all sites. We collected all environmental and host-pathogen data within a month, minimizing potential seasonality effects [22], [46]. We compared mean air temperatures from Glens Falls, NY, during the host (19.67 ± 1.66°C SD) and environmental sampling periods (21.78 ± 1.72°C SD), and found that the ranges of environmental temperatures highly overlapped during the month-long study period.

Statistical Analyses

To control for the effects of spatial autocorrelation among ponds, we analyzed our data using conditional autoregressions (CAR). We used CAR to test the relationship of each explanatory variable with *Bd* prevalence or infection intensity. We then used model selection tests including all biological and environmental variables and their interactions to find the combinations of variables that best explained *Bd*. Competing models were ranked based on Akaike Information Criterion (AICc), and we reported the model with the highest goodness-of-fit for each run. We also used CAR to test for associations of natural vegetation with host population (i.e., host average body

weight, capture rate) and community attributes (i.e., host community diversity, overall community biomass).

We used path analyses to statistically test for the strength of unidirectional cascading effects linking natural vegetation, canopy density, water temperature, and *Bd* infection parameters. Because canopy density may be a better proxy for microclimate than our actual temperature records from a single data logger per pond, we tested an alternative path diagram in which canopy density directly affected *Bd* infection parameters. We compared goodnessof-fit among models using Expected Cross-validation Index (ECVI), an AIC-based index. We conducted CAR using Spatial Analysis in Macroecology v4.0 [47] and path analyses using Systat v.10.1 [48].

Results

We detected *Bd* at all study sites with mean prevalence of 24.25% ± 16.41 SD and mean infection intensities of 29.36 ± 151.60 SD, reaching a maximum load of 1063.23 g.e. in our focal species without observed mortalities. Canopy density was the best predictor of *Bd* prevalence in *L. clamitans* (β_{CAR} = 0.765, P = 0.001, Figure 3.1a), followed by water temperatures [daily average temp. (β_{CAR} = 26.125, P = 0.004), maximum daily average temp. (β_{CAR}=25.187, P= 0.004), minimum daily average temp. (β_{CAR} = 26.000, P = 0.010)], and natural vegetation (β_{CAR} = 0.454, P = 0.021). We found no significant relationships between *Bd* prevalence and average body weight, capture rate, elevation, host community diversity, or overall host community biomass.

We found similar results for *Bd* infection intensity. Canopy density best predicted *Bd* infection intensities (β_{CAR} = 0.015, P < 0.001, Figure 3.1b), with

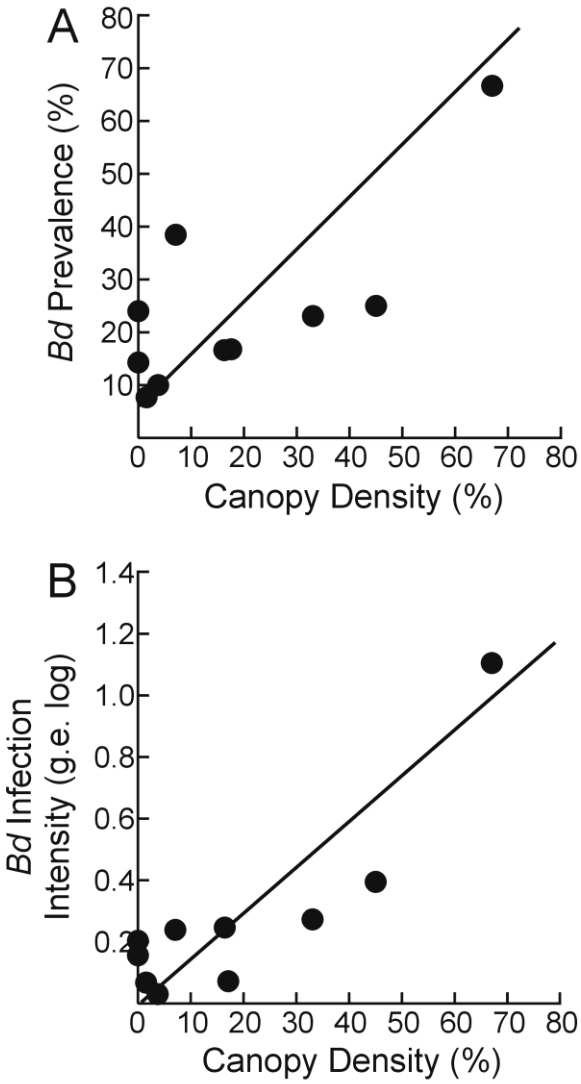

Figure 3.1: Effect of canopy density on *Bd* across populations of *L. clamitans* from the Adirondack region, New York, USA. (a) *Bd* prevalence; (b) *Bd* infection intensity.

populations in areas of closed-canopy having higher pathogen loads. We found that water temperatures [daily average temp. (β_{CAR} = 20.118, P = 0.001), maximum daily average temp. (β_{CAR} = 20.097, P = 0.002), minimum daily average temp. (β_{CAR} = 20.118, P = 0.003)], natural vegetation (β_{CAR} = 0.009, P = 0.007), average body weight (β_{CAR} = 20.011, P = 0.019), and capture rate (β_{CAR} = 0.110, P = 0.050) were also significant predictors of *Bd* infection intensity in *L. clamitans*. Similar to prevalence, we found no direct associations of *Bd* infection intensity with elevation, host community diversity, or overall host community biomass.

Looking simultaneously at all environmental and biological factors explaining *Bd* prevalence and infection intensity, our model selection identified three key environmental factors: canopy density, natural vegetation, and daily average water temperature (Table S3.1). The best model explaining *Bd* prevalence included only canopy density as a positive predictor (Table 3.1). The best model explaining *Bd* infection intensity included natural vegetation as a positive predictor, daily average water temperature as a negative predictor, and the interaction between those two variables (Table 3.1).

We tested for cascading effects among the environmental variables with highest explanatory power for *Bd* infection dynamics (Table 3.1, Table 3.2) and found that high canopy density resulted in lower water temperature (i.e., daily average temp.), which in turn predicted higher *Bd* prevalence (ECVI =2.405; Confidence Interval = 1.90, 4.07) and infection intensity (ECVI = 2.641; CI = 1.90, 4.478; Figure 3.2). Thus, habitat change strongly affected patterns of infection dynamics in our temperate amphibian populations, in that frogs in ponds surrounded by natural vegetation showed

Table 3.1. Conditional autoregressive models (CAR) simultaneously testing the effects of natural vegetation, canopy density, and water temperature on *Bd* prevalence and infection intensity in amphibian populations from the Adirondack region, New York, USA.

Term	β_{CAR}	***Std. coeff.***	***SE***	***t***	***P***
Prevalence					
Constant	*8.966*	*0*	*44.508*	*0.201*	*0.846*
Canopy density	**0.765**	**0.999**	**0.143**	**5.363**	**0.001**
Infection Intensity					
Constant	*4.189*	*0*	*0.413*	*10.136*	*<.001*
***i)* Natural vegetation**	**0.045**	**5.223**	**0.005**	**8.826**	**<.001**
***ii)* Water temperature - daily average**	**-0.179**	**-1.538**	**0.016**	**-11.066**	**<.001**
i)* × *ii)	**0.002**	**5.592**	**<.001**	**8.300**	**<.001**

Whole-model tests: prevalence: ($F = 7.418$, $n = 10$, $r^2 = 0.481$, $P = 0.026$); infection intensity: ($F = 38.376$, $n = 10$, $r^2 = 0.950$, $P < 0.001$). Std. coeff. stands for standard coefficient. Final models chosen based on Akaike Information Criterion (AICc).

Table 3.2. Best explanatory variables predicting *Bd* prevalence (%) and infection intensity (average g.e. log) across ten sites in the southern Adirondack region, New York, USA [Habitat loss (%), canopy density (%), and average water temperature (°C)].

Site	Lat.	Long.	*Bd* prevalence	*Bd* Infection Int.	Habitat Loss	Canopy Density	Average Water Temp.
1	43.138	-74.921	24.000	0.204	98.394	0	25.377
2	43.060	-74.856	38.462	0.239	88.836	7.083	21.775
3	43.362	-74.587	66.667	1.105	5.269	67.014	17.052
4	43.472	-74.414	7.692	0.068	98.659	1.563	20.635
5	43.016	-74.674	25.000	0.395	9.035	45.023	20.583
6	43.391	-74.719	14.286	0.156	87.066	0	24.259
7	43.390	-74.774	10.000	0.031	88.791	3.750	25.238
8	43.348	-74.617	16.667	0.247	44.683	16.443	21.798
9	43.301	-74.565	16.667	0.072	49.132	17.113	24.002
10	43.392	-74.544	23.077	0.273	46.856	33.073	19.827

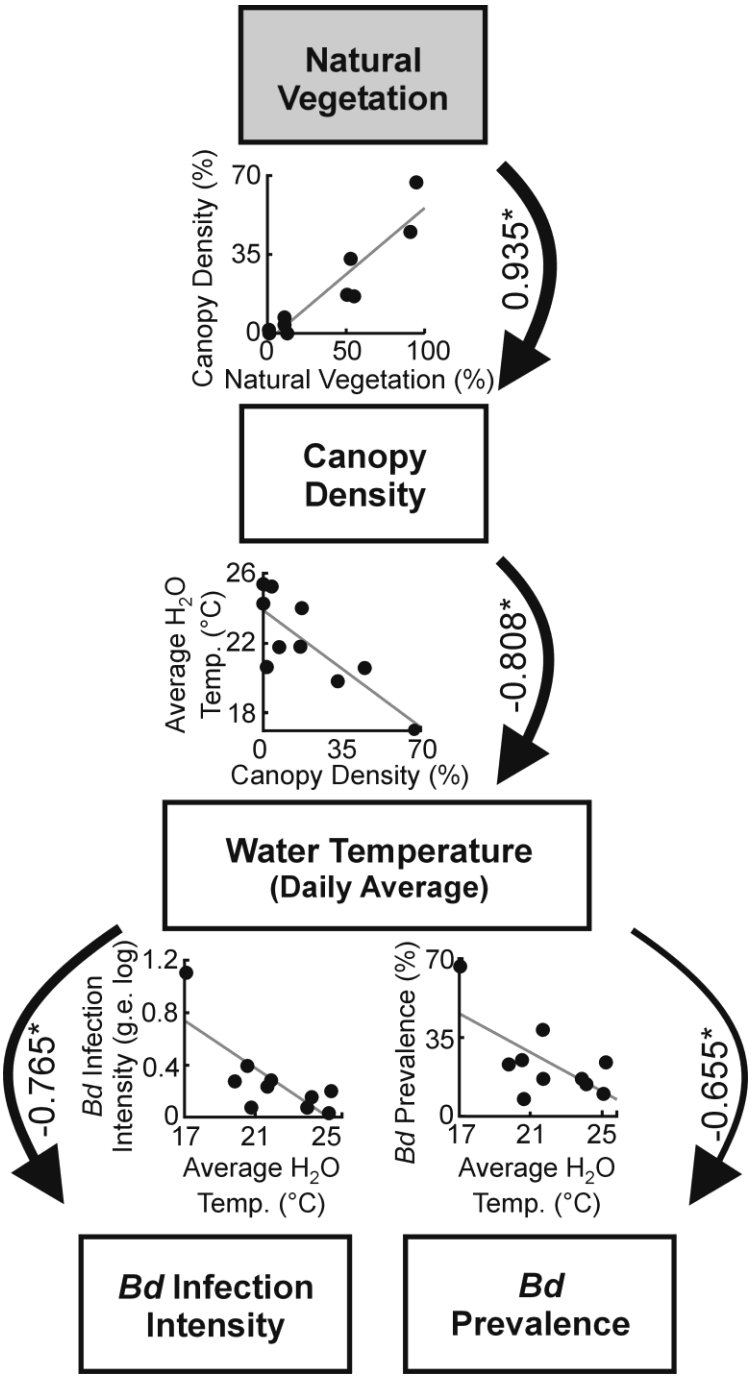

Figure 3.2: Path analyses indicating a unidirectional causal relationship between natural vegetation, canopy density, water temperature, and *Bd*. The relative strength of each effect is indicated by line width. Linear regressions are shown for each relationship. Numbers are standardized path coefficients (*P < 0.05). Diagram shows models for *Bd* prevalence and infection intensity combined.

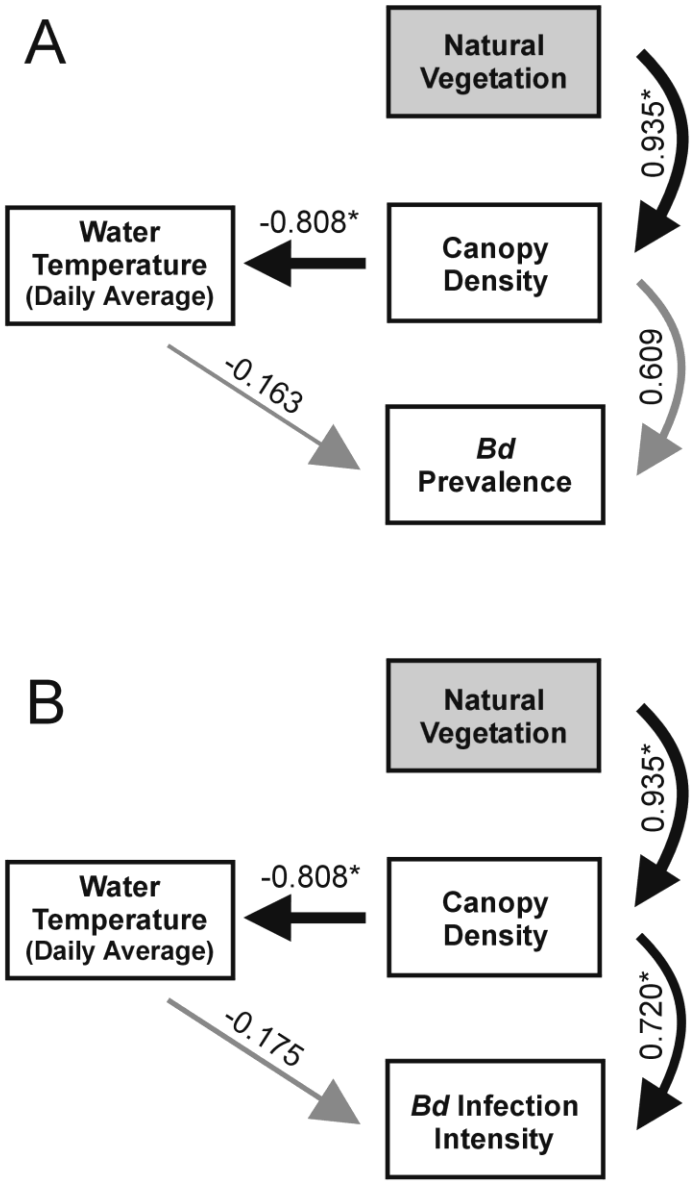

Figure 3.3: Alternative path models, including both direct and indirect effects of canopy density on *Bd*. (a) *Bd* prevalence; (b) *Bd* infection intensity. The relative strength of each effect is indicated by line width. Grey lines stand for non-significant effects. Numbers are standardized path coefficients (*P < 0.05).

higher *Bd* prevalence and infection intensity. In the alternative path models, including both direct and indirect effects of canopy density on *Bd* parameters, we found that canopy density was a direct positive predictor of *Bd* infection intensity (ECVI = 2.290; CI = 2.00,3.72; Figure 3.3b), but not a significant predictor of *Bd* prevalence (ECVI =2.371; CI =2.00, 3.87; Figure 3.3a). Both the strictly unidirectional diagram (Figure 3.2) and the alternative models including direct effect of canopy density on *Bd* (Figure 3.3) showed the same goodness-of-fit according to ECVI. These results corroborate earlier findings in both tropical [6] and temperate [23] amphibian populations.

The high evenness and low diversity among our sampling ponds (D= 0.840 ± 23 SD) underscores the dominance of the focal species across this landscape (i.e., *L. clamitans*: N=166, *L. catesbeianus*: N= 16, *L. palustris*: N= 2, and *L. septentrionalis*: N= 1). The amount of natural vegetation surrounding our sampling ponds did not significantly predict host population and community attributes; namely host average body weight (β_{CAR} = 0.344, P = 0.145), host capture rate (β_{CAR} = 20.024, P =0.175), overall host community biomass (β_{CAR} < 0.001, P = 0.119), species richness (β_{CAR} = 0.009, P = 0.215), and host diversity (β_{CAR} = 20.002, P = 0.399).

Discussion

The effects of habitat loss and forest fragmentation on host-pathogen interactions are varied [1–3], [5], [6], and depending on the mechanisms underlying disease dynamics habitat change can either increase or decrease disease risk. A shift in microclimate is sometimes the leading mechanism controlling disease dynamics [5]. Our data confirm that microclimate shifts arising from disturbance of natural

vegetation play an important role in amphibian host-pathogen interactions in temperate systems. Our path analyses underscore the importance of differences in temperature associated with canopy density as a likely driver of amphibian disease risk in natural forests, corroborating a pattern reported earlier in tropical [6] and temperate systems [23]. Host community attributes did not play an important role in our study system, a result that is not surprising given the high dominance of *L. clamitans* and low amphibian diversity of the eastern forest-boreal transition [34], [37]. Our sampling period excluded two local species that breed earlier in the spring when temperatures are cooler (i.e., *Pseudacris crucifer* and *L. sylvaticus*). Although these species may help maintain *Bd* throughout the cold months, it is unlikely that they have a strong effect on disease dynamics later in the season, because they spend long periods of time away from water bodies [49] and breed during periods of suboptimal temperature conditions for *Bd* growth [32]. Therefore, it is not surprising that both species show low *Bd* infections in the wild [37]. Overall, our results show that vegetation cover influences *Bd* prevalence and infection intensity in temperate amphibian populations by modulating shade and associated microclimatic patterns, and that host community structure plays at most a minor role in our study system.

The best predictors of *Bd* prevalence and infection intensity were environmental variables associated with vegetation cover and microclimate (see Table 3.1). The amount of natural vegetation at the water line is strongly associated with the proportion of shade at the edge of the pond. Thus, the amount of canopy density directly affects air and water temperatures and serves as a proxy for the thermal conditions at frog basking sites. Canopy cover also regulates the availability of

shallow, warm-water patches in which amphibians might reduce or clear *Bd* infections. Our path analyses confirm that canopy density indirectly controls both *Bd* prevalence and infection intensity through these changes in pond thermal profiles. We also found equal support from a model where canopy density directly affects *Bd* infection intensity (Figure 3.3b). A study on *Bd* infection levels in red-spotted newts (*Notophthalmus viridescens*) in Pennsylvania showed that the proportion of leaf-litter and vegetation in the pond substrate correlated positively with *Bd* prevalence and infection intensity [23]. The authors propose that leaf-litter and emergent vegetation might increase *Bd* transmission by providing substrates for *Bd* growth. Alternatively, leaf-litter may be an indicator of canopy cover and total degree of shade, which are potentially the true drivers of higher *Bd* levels in natural forest habitats. Similarly, in Costa Rica and Australia, localities with little to no canopy cover may provide amphibians with a refuge from *Bd*-induced extinction [50], [51] presumably due to similar mechanisms related to micro environmental controls. Two rainforest frog species that suffered severe declines in Australian rainforests have been rediscovered in open dry forest sites coexisting with high prevalence of *Bd* year round (above 69%) [50]. Our results corroborate these recent findings [23], [50], [51] and show that modulation of *Bd* infections by environmental factors is a general phenomenon, and that these environmental controls linking natural vegetation to *Bd* dynamics are similar in tropical and temperate amphibian communities.

Microclimate can affect amphibian disease risk in two ways by (i) regulating *Bd* growth and persistence in both host and the environment, and (ii) by changing host's ability to fight and clear *Bd* through thermoregulation. The growth rate of *Bd* in

the laboratory is strongly temperature-dependent, with an optimum climatic envelope ranging between 17–25°C and reduced persistence at temperatures above 28°C [32]. Average maximum water temperatures across all our sampling ponds (i.e., 24.41°C ± 63.12 SD) fell near the upper threshold of this microclimatic envelope, indicating that *Bd* growth may have been limited during some periods in our highest-temperature ponds. In fact, in four of our ponds 40.85°C ± 4.42% SD of water temperature records were above 25°C during the study period. Our data closely match the association between *Bd* infections and temperature found by Raffel et al. [23], with low *Bd* infection intensities and prevalence at sites with average water temperatures above 23°C. In the State of Maine, USA, *Bd* infections were lower during the peak of the summer than in spring and fall, presumably because water temperatures often exceeded the optimal temperature range for *Bd* growth [37]. In the State of Arizona, *Bd*-infected frogs were largely absent from sites where spring water exceeded 25°C [52]. Additionally, laboratory experiments with an Australian *Bd* strain showed that pathogen growth in vitro is hampered by short exposures (i.e., one hour daily) to high temperatures that would typically be available to frogs at basking sites [53]. Some amphibians may rely on behavioural fever to fight *Bd* infections [54], therefore warmer and drier microclimates may decrease the odds of both *Bd* infection and transmission in open habitats [55]. At cooler temperatures, amphibian hosts may also lose the ability to mount antimicrobial responses, which translate in higher *Bd* loads [56]. In the Sierra Nevada, however, *Bd* infection intensity and frog survival were unrelated to water temperature [57], but the maximum temperature at the three focal high elevation sites rarely exceeded 25°C.

Our results indicate that the small-scale effects of vegetation and microclimate on our host-*Bd* system are larger than the effects imposed by density-dependent forces that typically predict prevalence and infection intensity in other temperate amphibians [58–60]. In a simple regression, capture rate positively predicted *Bd* infection intensity, potentially indicating density-dependent controls; however, this effect was marginal and capture rate was not a significant predictor of *Bd* when considered together with environmental factors in model selection. The potential effect of density on *Bd* was not linked to forest cover because we did not detect a significant effect of natural vegetation on host capture rate. Pathogen build-up to lethal infection intensities is more likely to occur in dense populations, under conditions that promote continuous reinfection of the hosts [59], [60]. Nonetheless, our focal species exhibited lower infection intensities than susceptible hosts in Sierra Nevada [58], [59], or persisted under host densities that might not trigger outbreaks. Future studies should investigate potential associations among vegetation type and long-term density-dependent factors of pathogen dynamics.

Earlier studies in both tropical and temperate zones have found ontogenetic differences in *Bd* susceptibility [22], [23]. In both cases, juveniles and sub-adults showed higher *Bd* prevalence and infection intensities. One potential explanation is that disease risk drops with age in response to host-acquired immunity, as repeated exposure to a given pathogen increases host resistance [28], [55]. In addition, a reorganization of host immune system occurs during metamorphosis, and postmetamorphic defenses may take some time to mature [28]. Although average body weight was in fact a negative predictor of *Bd* infection intensity when considered

independently, this parameter became a weak predictor when considering other environmental variables in the analysis. This weak effect of host body weight on *Bd* infection coupled with the fact that vegetation cover had no influence on host capture rate is an indication that, in our study system, habitat change has a larger influence on pathogen fitness than on host fitness. This result suggests that our focal species is highly resistant to *Bd* regardless of microclimate and vegetation. In fact, *L. clamitans* persists with a local *Bd* strain in the laboratory within optimal *Bd* grow temperatures [61].

We have shown that disturbances to natural forest habitats reduce *Bd* infections in both temperate and tropical systems [6], which could mislead some decision makers to propose forest removal as an amphibian conservation strategy. However, habitat loss alone is the leading factor driving amphibian extinctions and declines worldwide [16], [17], [38], [62], thus intentional habitat disturbances will not serve as a strategy to prevent biodiversity loss due to wave-like *Bd* epidemics [63]. Fortunately, there are promising conservation strategies that do not include habitat alteration. For example, captive breeding of frogs with high immunogenetic *Bd* resistance or tolerance could be a useful tool for assisted reintroductions in the wild [64], and would be especially promising in areas of pristine rainforests where *Bd* is most prevalent. With the high rate of anthropogenic modification to temperate and tropical forests, understanding how vegetation cover and disease interact is critical for predicting *Bd* spread and developing appropriate management tools for wild populations. Our results indicate that species-specific in situ management strategies will need to consider fine-scale

microclimatic factors to safeguard *Bd*-susceptible species with narrow geographic distributions [38] outside areas of climatic refugia [65].

Acknowledgments

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CHAPTER 4

PARTITIONING THE NET EFFECT OF HOST DIVERSITY ON AN EMERGING AMPHIBIAN PATHOGEN

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Abstract

The 'dilution effect' hypothesis predicts that diverse host communities will show reduced disease. The underlying causes of pathogen dilution are complex, because they involve non-additive (driven by host interactions and differential habitat use) and additive (controlled entirely by host species composition) mechanisms. Here, we used measures of complementarity and selection traditionally employed in the field of Biodiversity-Ecosystem Function (BEF) to quantify the net effect of host diversity on disease dynamics of the amphibian-killing fungus *Batrachochytrium dendrobatidis* (*Bd*). Complementarity occurs when average infection load in diverse host assemblages departs from that of each component species in uniform populations. Selection occurs when diversity-disease relationships are disproportionately impacted by one or few host species. We experimentally infected tropical amphibian species of varying life-histories, in single- and multi-host treatments, and measured individual *Bd* infection loads. Host diversity reduced *Bd* infection in amphibians through a mechanism analogous to complementarity (*sensu* BEF), potentially by reducing shared habitat use and transmission among hosts. Additionally, the selection component indicated that one particular terrestrial species showed reduced infection loads in diverse assemblages at the expense of neighboring aquatic hosts becoming heavily infected. By partitioning components of diversity, our findings underscore the importance of non-additive mechanisms underlying the dilution effect.

Introduction

Biodiversity loss is happening at increasingly rapid rates and changing the

distribution of organisms around the globe (Pimm et al. 1995). Biodiversity declines alter several features of communities, including the number of species (species richness), identity of species (species composition), their relative abundances (species evenness), and species interactions (Chapin et al. 2000). These features, in concert, drive key mechanisms responsible for ecosystem functioning, such as primary production, competition, predation, and disease dynamics (Tilman 1999, Daszak et al. 2000, Ostfeld et al. 2009, Johnson & Thieltges 2010, Pagan et al. 2012).

A number of recent studies have shown that declines in biodiversity can lead to increases in disease risk (Dobson et al. 2006, Keesing et al. 2006, Ostfeld & Keesing 2012, Pagan et al. 2012), but the underlying mechanisms leading to this pattern, and their generality, are complex and controversial (Randolf & Dobson 2012, Wood & Lafferty 2013, Ostfeld & Keesing 2013). This phenomenon of high host diversity reducing disease, termed the *dilution effect* (DE), can arise through several potential mechanisms by which diversity affects transmission, including encounter reduction, susceptible host regulation, infected host mortality, and recovery augmentation (reviewed in Keesing et al., 2006). These mechanisms have in common that both host interactions and the identity of the species influence the likelihood of transmission and disease. Encounter reduction − one clear mechanism that lowers transmission rates and thus leads to DE − operates in communities in a couple of ways; first, the proportion of susceptible and immune hosts may change with shifts in diversity, leading to reduced encounter rates between infected and uninfected hosts (Keesing et al. 2006). Second, diverse host communities may partition niche space more finely due to local adaptation, specialization, and competition (MacArthur & Levins 1967), and may in

turn experience reduced encounter rates and transmission among conspecifics, and pathogen spillover across host species (Ostfeld et al. 2009). Identifying the drivers of transmission most affected by biodiversity loss and their relative contribution to wildlife diseases has been challenging, despite their importance for effective wildlife management and disease forecasting. Without a clear understanding of these mechanisms we will not be able to predict general patterns of disease dynamics in nature.

The mechanisms behind diversity-disease relationships are in many ways parallel to other important processes driving ecosystem functioning or performance (Tilman et al. 1997, Loreau & Hector 2001, Cardinale et al. 2007). Studies in the field of Biodiversity-Ecosystem Functioning (BEF) demonstrate that a decrease in species diversity can reduce primary productivity (Loreau & Hector 2001, Cardinale et al. 2007, Cook-Patton et al. 2011) and increase herbivory (McArt & Thaler 2013) through a variety of additive and non-additive mechanisms. Within the BEF literature, additive mechanisms are those driven by host composition, such that the ecological response of any species in a diverse assemblage can be predicted by its response in monoculture and its relative abundance in the mixed community (Hughes et al. 2008). Additive mechanisms also apply to diversity-disease relationships (Ostfeld et al. 2009), and as defined, a necessary condition is that host species will respond identically to disease in single-host and mixed assemblages. The sampling effect is a common additive mechanism that applies to both BEF and DE (Loreau & Hector 2001, Johnson & Thieltges 2010). It states that diverse host assemblages have a higher probability of

including a species that strongly affects ecological responses such as primary productivity (Huston 1997, Tilman et al. 1997) and/or pathogen transmission.

While additive mechanisms almost certainly play a role in disease dynamics, many important mechanisms for BEF and diversity-disease relationships are non-additive. Non-additive mechanisms occur when the ecological response of a given species in mixed assemblages cannot be predicted by how it responds in uniform populations. A non-additive mechanism commonly identified in the field of BEF arises due to interspecific differences in resource utilization with downstream effects on primary productivity (Loreau & Hector 2001). Non-additive mechanisms also apply to diversity-disease relationships (Ostfeld et al. 2009), and can result from host interactions and differential habitat use to alter disease response of host species in diverse assemblages. Two non-additive ecological mechanisms that have been widely studied in BEF, facilitation/inhibition and niche partitioning, are jointly referred to as 'complementarity' (Cardinale et al. 2007). In facilitation/inhibition, heterospecific neighbors control damage to a particular plant species by attracting or repelling herbivores (e.g., associational susceptibility/resistance) (Barbosa et al. 2009). In niche partitioning, species have complementary habitat use or resource utilization, and thus diversity often has a positive influence on primary productivity (Tilman 1999). Although complementarity clearly applies to BEF studies on primary productivity, its potential role in DE is not intuitive, because host species do not complement each other in order to obtain higher or lower infection loads. However, parallel processes do exist. For example, differential habitat use among host species can affect disease risk if species diversity causes a reduction in niche overlap and thereby decreases host contact

rates and transmission. Furthermore, the presence of a particular species may increase or decrease the probability of infection in the whole host community through direct association; this mechanism would be equivalent to facilitation/inhibition.

In addition to complementarity, selection is a second component of diversity that can potentially affect diversity-disease relationships (Ostfeld et al. 2009). Selection can occur when positive or negative diversity-disease relationships are exacerbated by the disproportionate impact of a particular host species (or guild) (Tilman et al. 1997, Loreau & Hector 2001). Even though selection is typically strongly driven by species composition (Huston 1997), it may also occur due to one species' ability to become disproportionately less infected (thus becoming locally dominant over multiple generations) at the expense of neighboring species becoming heavily infected (and becoming locally extinct) (Loreau & Hector 2001). Therefore, selection does not depend just on initial species frequencies (additive mechanism), but also on the effects of host interactions (non-additive mechanism; Loreau & Hector 2011).

Here, we used an amphibian host-pathogen system to identify mechanisms underlying diversity-disease relationships. We partitioned the net effect of host diversity on pathogen infection loads, and measured the relative contribution of 'complementarity' and 'selection' to disease risk. We experimentally exposed tropical amphibians to a panzootic strain of the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), in single-host and multi-host treatments. This epidermal pathogen has a broad host range among amphibians (Olson et al. 2013) and is implicated in population declines and species extinctions worldwide (Berger et al. 1998; Lips et al.

2008; Alford & Rowley 2008; Wake & Vredenburg 2008). We used seven wild-collected tropical amphibian species that fall along a continuum of breeding mode and habitat use, ranging from fully terrestrial to mostly aquatic. Our specific goals were to (1) test whether the mechanisms of complementarity and selection, or a combination of both, drive diversity-disease relationship in our study system, and (2) identify the contribution of species composition to pathogen dynamics. Our work demonstrates the application of principles of BEF to disease ecology. This perspective offers an accurate and fine-scale measurement of the effects of biodiversity on disease and enhances our mechanistic understanding of diversity-disease outcomes. Both of these goals are increasingly critical with the rapid anthropogenic acceleration of biodiversity loss.

Methods

Host species

We captured adult anurans of seven locally abundant species in October 2012 from Parque Estadual da Serra do Mar – Núcleo Santa Virginia in the Brazilian Atlantic Forest (-23.35° S, -45.16° W). We assigned host aquatic index (AI) to each species (modified from Lips et al. 2003) which quantifies amount of time spent in aquatic environments summed across different amphibian life stages. Because *Bd* is a waterborne fungal pathogen, AI also serves as a relative measure of species-specific exposure and transmission probability in natural communities (Kriger & Hero 2007, Lips et al. 2006). Our seven focal host species ranged from exclusively terrestrial species (i.e., direct developers) occupying forest leaf-litter [(AI=0) *Brachycephalus pitanga* (PIT) and *Ischnocnema parva* (PAR); Brachycephalidae], to species breeding

in aquatic habitats but occupying the arboreal stratum [(AI=1) *Dendropsophus minutus* (MIN), *Scinax hayii* (HAY), and *Hypsiboas bandeirantes* (BAN); Hylidae], to species breeding in aquatic habitats and occupying the margins of streams and other bodies of water [(AI=2) *Physalaemus cuvieri* (CUV), Leptodactylidae; *Hylodes phyllodes* (PHY), Hylodidae]. Thus our focal taxa represent a full gradient of host aquatic index found in the natural environment ranging from terrestrial (AI=0) to highly aquatic (AI=2).

Focal pathogen

We investigated the effect of host diversity on dynamics of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which has caused population declines in hundreds of amphibian species in the Neotropics (Berger et al. 1998; Lips et al. 2008), North America (Vredenburg et al. 2010), Europe (Walker et al. 2010), and Australia (Berger et al. 1998; Alford & Rowley 2008). *Bd* can be transmitted by frog-to-frog contact, but it is also spread through host contact with aquatic environmental reservoirs (Rowley & Alford 2007). Because many amphibian species share the same aquatic breeding sites (e.g., streams, ponds), interspecific transmission is likely, as free-living zoospores can be acquired from the water and initiate the infection of amphibian skin (Longcore et al. 1999). Therefore, the mode of transmission could influence process in both BEF and disease dynamics. For the present experiment, we used a global panzootic *Bd* strain (CLFT023) isolated from the Atlantic Coastal Forest, State of Minas Gerais (Schloegel et al. 2012).

Experimental design

Each experimental unit consisted of a rectangular terrarium (40 x 29 x 13.5 cm) with terrestrial habitat at one end of the container (autoclaved moist *Sphagnum*) and aquatic habitat at the other end. To control for host density, each of our experimental units (single-host or multi-host) included four individual amphibians. We replicated single-host treatments four times for each of the seven host species, totaling 28 experimental units. We randomly assigned four unrepeated host species (species richness = 4) to each multi-host treatment, totaling 25 unique host assemblages.

To clear potential *Bd* infections from the field we treated experimental animals with Itraconazole (0.01% solution) for 7 days (Pessier & Mendelson 2010) prior to beginning the experiment. A randomly selected subsample of hosts (N = 40) tested negative after the clearing protocol. For the experimental infection, we cultured *Bd* strain CLFT023 (Schloegel et al. 2012) in tryptone agar Petri plates at ~19°C for 7d. We harvested *Bd* by flooding plates with distilled water and waiting for ~3 h for zoospores release. We then pooled inoculum from plates, quantified zoospores with a hemocytometer, and added 10^6 zoospores in 250 ml of dechlorinated water to the wet end of each experimental unit. This protocol ensured comparable exposure across replicates. We added the amphibians to the terrestrial habitat of each experimental unit and kept temperatures at 19.74°C ± 0.55 SD on a 12 h day-night light cycle.

We monitored amphibians daily and fed them pinhead crickets (*Gryllus* cf. *assimilis*) *ad libitum*. We swabbed all individuals and terminated the experiment on the 18th day post-infection. This period encompasses approximately five replication cycles of *Bd* (Longcore et al. 1999) and is sufficient for the pathogen to reach peak infections

in susceptible amphibians (Longo et al. 2010, Savage & Zamudio 2011). During the course of the experiment, we swabbed dead or dying animals and removed them from the experimental units. We tested swabs for *Bd* in duplicate using Taqman qPCR (Boyle et al. 2004; Hyatt et al. 2007) with standards of 0.1, 1, 10, 100, and 1000 zoospore genomic equivalents (GE) to determine the infection intensity of *Bd* in each individual host.

Partitioning the effects of host diversity on Bd

A seminal paper by Loreau & Hector (2001) described a quantitative framework for calculating the contribution of selection and complementarity to the net diversity effect on primary producers. This framework has been widely used in BEF studies to examine the impact of plant diversity on yields and herbivory (Cardinale 2007; Cook-Patton et al. 2010; McArt & Thaler 2013); to our knowledge, this study is its first application to disease ecology. The net diversity effect (here redefined as net effect of host diversity) is calculated using the equation: $\Delta Y = N\overline{\Delta RY}\,\overline{M_i} + N\mathrm{cov}(\Delta RY, M)$, where $N\overline{\Delta RY}\,\overline{M_i}$ measures complementarity, $N\mathrm{cov}(\Delta RY, M)$ measures selection, N = number of host species in the multi-host assemblages, M_i = *Bd* infection loads of species *i* in single-host treatments, and $\Delta RY = RY_{Oi} - RY_{Ei}$ = the deviation from expected relative *Bd* infection loads of species *i* in the multi-host assemblage (Loreau & Hector 2001).

The complementarity component measures changes in the average infection in diverse host assemblages relative to weighted average loads of host species in uniform populations. Negative values imply that average infection loads are lower in multi-host

assemblages than predicted by the infection loads of each component host species in single-host systems. Positive values indicate that average infection loads are higher in multi-host assemblages than predicted by the infection loads of each component species in single-host systems.

Selection, so named because its application is based on Price's general theory of selection, is measured by a covariance function (Price 1970). In disease ecology, strong selection can occur when relative infection loads of particular species shift in one direction at the same time that relative infection loads of neighboring species respond in the opposite direction. Therefore, selection can occur when positive or negative diversity-disease relationships are leveraged by the disproportionate impact of a particular species (or guild) to disease. Negative values for selection indicate that host species, normally carrying high infection loads in uniform populations, obtain disproportionately lower loads while in diverse assemblages. This happens at the same time that species normally carrying low infection loads in uniform populations obtain higher loads in diverse assemblages. In contrast, positive selection values indicate that species normally carrying high infection loads in uniform populations obtain even higher infection loads in multi-host assemblages at the same time that other species in the community become less infected. Selection and complementarity add up to the net effect of host diversity; in this paper we maintain this terminology for consistency across fields.

Statistical analyses

We compared average *Bd* infection intensity ($\log_{10}$ transformed) between diversity treatments (single-host and multi-host) and among the three categories of host aquatic index using a standard least square General Linear Model (GLM). We used stratified models with individuals nested within experimental units (nested ANOVA; Sokal & Rohlf 2000, SAS 2012). We tested whether selection, complementarity, and the net effect of host diversity were positive, negative, or neutral by observing whether 95% confidence intervals overlapped zero. In addition, we used a *t*-test to compare average host aquatic index (AI) between assemblages showing positive and negative complementarity or selection. We did not analyze prevalence data because >96% of the hosts became infected with *Bd* during the experiment. Mortality was low in both single-host (N=3) and multi-host treatments (N=6). Nonetheless, because mortality affects total host densities, which was otherwise controlled in our experiment, we repeated the analyses and quantified complementarity, selection, and the net host diversity effect while excluding assemblages that experienced mortality.

Results

Bd infection loads were reduced by 66.5% in multi-host compared to single-host treatments (multi-host: Least Square Mean = 61.892 zoospore genomic equivalents (GE); 1.171 logGE; single-host: LSM = 184.612 GE; 1.354 logGE; F=4.434, $P < 0.039$; Figure 4.1a). We found that this significant reduction in *Bd* infection loads in diverse host assemblages was driven by the complementarity component (Figure 4.1b). Our measures of selection, however, showed both positive

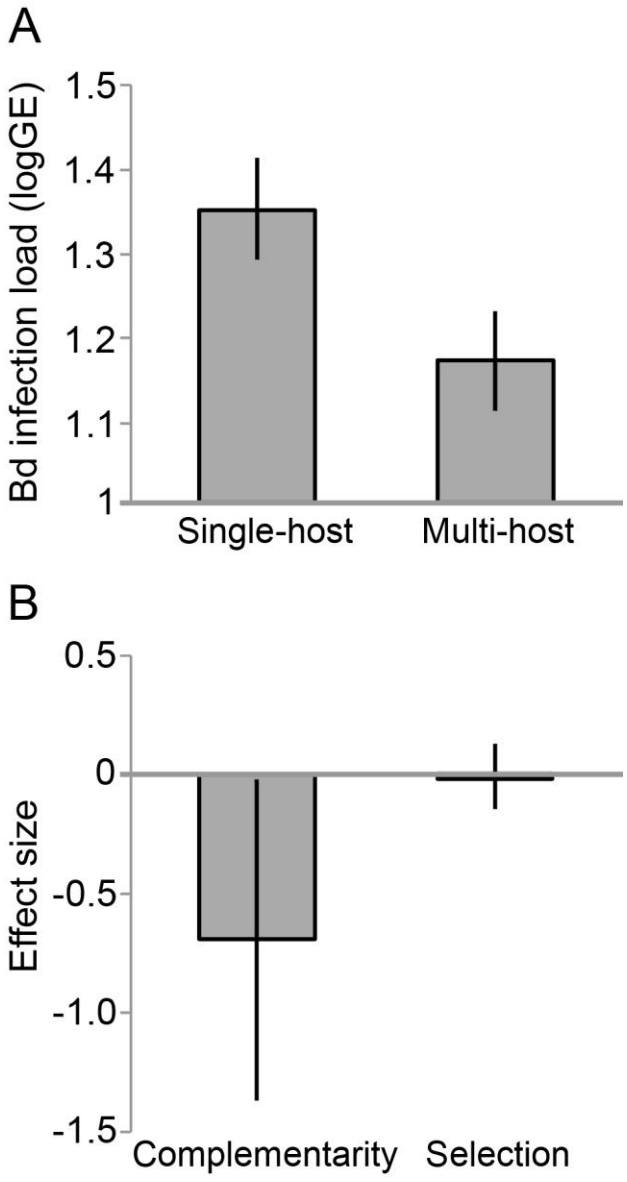

Figure 4.1: Effects of host diversity on *Bd* infection loads. Average *Bd* infection loads in single-host and multi-host treatments (mean ± 95% SE; a). Net effect of host diversity partitioned into the two components complementarity and selection (b).

and negative values (of lower intensity) across our mixed host assemblages (Figure 4.1b). Combined, complementarity and selection resulted in a net effect of host diversity reducing *Bd* infection loads (Mean = -0.695 logGE; -0.040,-1.351 CI). These results remained unaltered after assemblages that experienced mortality were omitted from calculations (Table S4.1).

As expected, host species with a high aquatic index carried higher *Bd* infection loads than terrestrial hosts independent of diversity treatment (F=11.288, P = 0.001; Figure 2). Furthermore, most host species showed a decrease in *Bd* infection loads in multi-host assemblages (Figure 4.2). Our test for the effect of host identity on the observed dilution effect showed no association between average host aquatic index and the strength of complementarity across diverse host assemblages (F = 0.003; P = 0.954). Conversely, host species composition significantly predicted the direction of selection. Specifically, multi-host assemblages where selection was positive were composed by species with lower host aquatic index when compared to assemblages where selection was negative (F = 8.267; P = 0.008; Fig 4.3). This pattern was strongly influenced by the terrestrial host *Brachycephalus pitanga,* which showed a decrease in infection loads as neighboring aquatic host species experienced pathogen amplification (Fig 4.3).

Discussion

Our experiment demonstrated that diversity can reduce *Bd* infection loads in amphibians through a non-additive mechanism that falls under the umbrella of complementarity (*sensu* BEF). Specifically, lower *Bd* transmission among host species

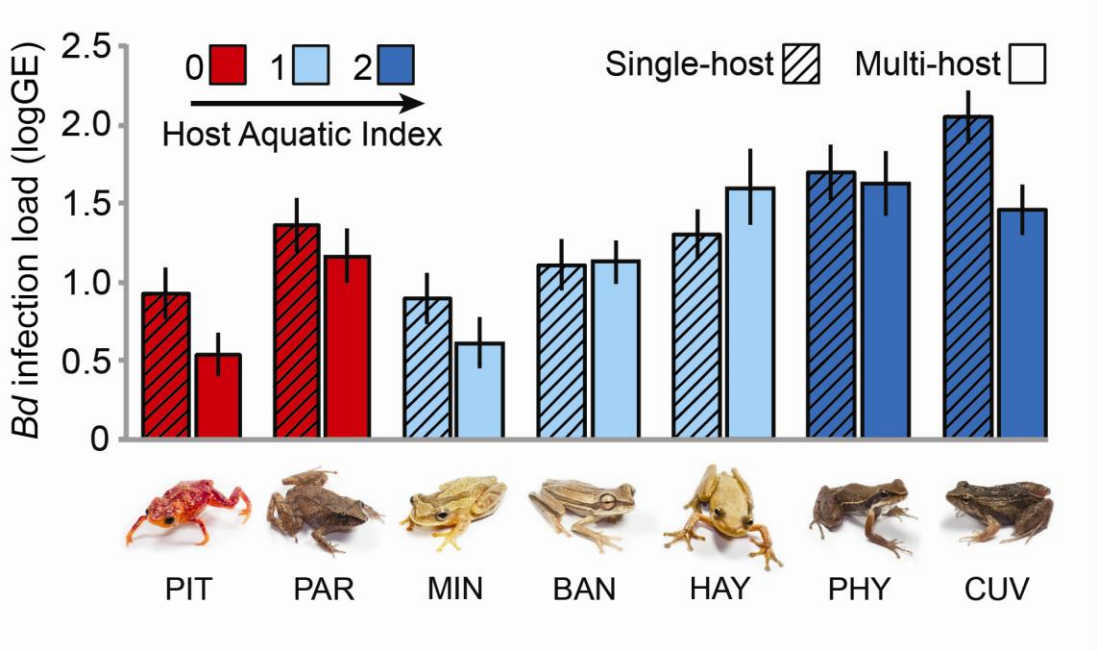

Figure 4.2: Average *Bd* infection loads across single-host and multi-host treatments (least square mean ± SE). Species acronyms are: *B. pitanga* – PIT, *I. parva* – PAR, *D. minutus* – MIN, *H. bandeirantes* – BAN, *S. hayii* – HAY, *H. phyllodes* – PHY, and *P. cuvieri* – CUV. Colors represent host aquatic index ranging from terrestrial (AI=0) to highly aquatic (AI=2).

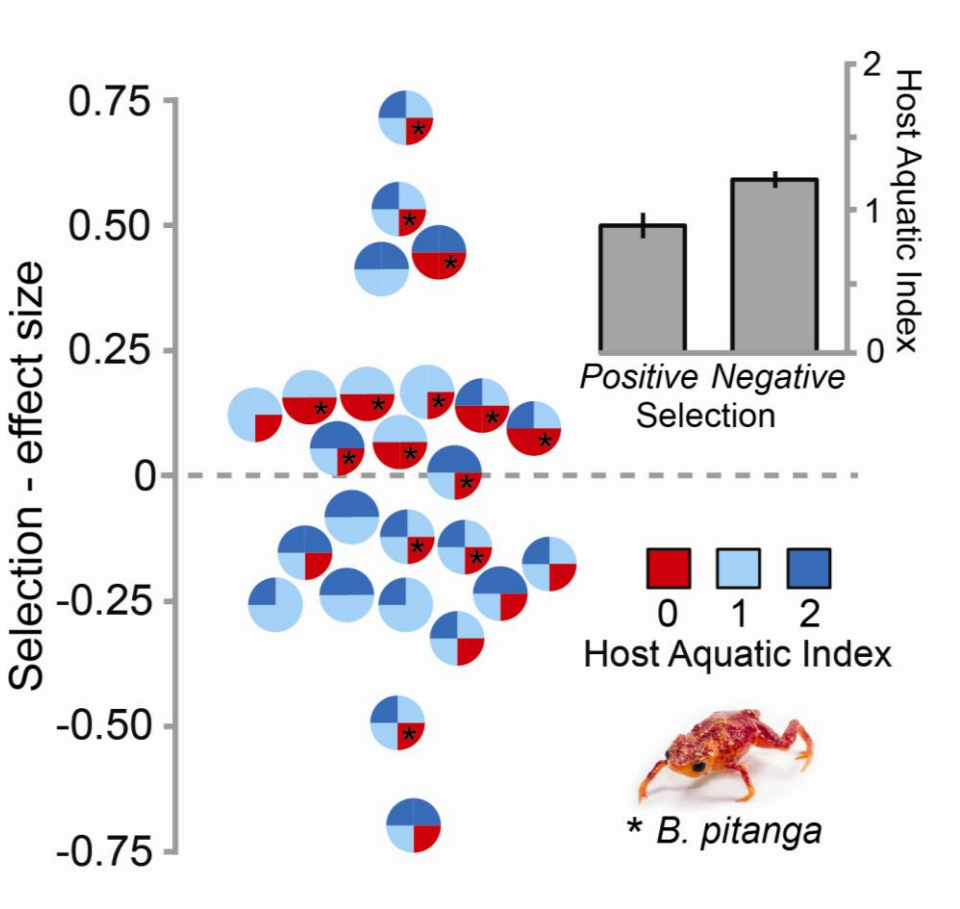

Figure 4.3: Selection component across 25 unique and equally diverse host assemblages. Bars represent average host aquatic index (± 95% SE) for assemblages experiencing positive and negative selection (graph jittering added to avoid overlapping data points). Assemblages containing the aposematic pumpkin toadlet (*Brachycephalus pitanga*) are highlighted with an asterisk. Colors represent host aquatic index ranging from terrestrial (AI=0) to highly aquatic (AI=2).

due to reduced shared habitat use was the likely mechanism leading to the observed DE at the community level. Furthermore, the highly variable selection component in our experiment indicated that the presence of a particular host species in diverse assemblages can disproportionately increase or decrease infection loads in neighboring hosts through direct association. Therefore, non-additive and additive mechanisms of diversity were tightly linked as drivers of chytridiomycosis in our experimental system.

Previous foundational work attributes the dilution effect to a combination of additive and non-additive mechanisms (Ostfeld & Keesing 2012). Nevertheless, isolating the effect of biodiversity *per se* from the impact of host species composition is often a challenging task in natural systems due to the correlative nature of field-collected data. In many cases, anthropogenic habitat change is the proximate force selectively removing host species with high degrees of ecological specialization, and thus, habitat generalist hosts often become dominant in depauperate communities (LoGiudice et al. 2003, Lee et al. 2008, Allan et al. 2009, Johnson et al. 2013). In the case of Lyme disease, biodiversity loss promotes dominance of the habitat generalist *Peromyscus leucopus*, a highly competent host of the pathogen *Borrelia burgdorferi* (LoGiudice et al. 2003). For West Nile virus infections, host diversity and community competence are tightly negatively correlated, such that depauperate host communities are dominated by competent reservoirs (Allan et al. 2009). Likewise, biodiversity loss increases transmission of the parasitic trematode *Ribeiroia ondatrae* because highly competent amphibian hosts dominate species-poor communities (Johnson et al. 2013). These studies found evidence for non-additive mechanisms (shifts in host species interactions) as well as a strong additive mechanism driving DE (numerical dominance

of a competent host species). In our study system with randomly assembled host communities, diversity was negatively associated with disease risk when total host density was controlled for. We expect that the DE we observed experimentally would be even stronger in the wild if biodiversity loss in a real systems simultaneously leads to changes in density and in community composition (e.g., by favoring superspreaders or species that induce continuous re-infection in neighboring host species). Even though our experimental study does not perfectly mimic the natural assembly and disassembly of amphibian communities, it provides a quantitative framework for the relative contribution of additive and non-additive components of diversity to disease dynamics.

By partitioning the net effect of host diversity, we quantified the relative contribution of complementarity and selection. The main mechanism leading the observed community-level DE falls under the umbrella of complementarity (*sensu* BEF; Loreay & Hector 2001), where diversity *per se* led to lower infection loads in diverse assemblages. Niche theory predicts that species in diverse assemblages will compete for resources such as space and thus benefit from reduced overlap in habitat use (MacArthur & Levins 1967). Therefore, lower niche overlap can cause both host encounter reduction and decreased exposure to the aquatic pathogen reservoirs such as *Bd* (reviewed in Keesing et al. 2006), thus having a potential impact on both density- and frequency-dependent *Bd* transmission (Dobson 2004, Rachowicz & Briggs 2007). A second potential mechanism by which complementarity can lead to DE is inhibition through associational resistance among host species (Barbosa et al. 2009). One example would be if a particular host species deters or repels pathogens from the

shared environment, thus lowering infection loads in the entire community. However, our community-level measures of *Bd* infection loads in diverse assemblages were not strongly driven by a particular species (or combination of species), as we did not find an association between host composition and the intensity of complementarity.

Even though host composition did not explain the intensity and direction of complementarity, we found it to be important in explaining selection. Specifically, we found positive values for selection in assemblages containing both terrestrial and aquatic hosts and negative values in assemblages dominated by aquatic hosts (Figure 4.3). The presence of the terrestrial aposematic pumpkin toadlet (*Brachycephalus pitanga*) was the likely cause of the observed variation in diverse host communities because this particular host carried lower infection loads in diverse assemblages (see Figure 4.2) as loads increased in neighboring aquatic hosts. *Brachycephalus pitanga* secretes tetrodotoxin (a potent neurotoxin) from their skin and it is possible that this species deterred more susceptible aquatic hosts from the dry terrestrial habitat, thus disproportionately increasing their exposure to *Bd* in the aquatic environment. Alternatively, pumpkin toadlets may compromise the ability of neighboring hosts to fight infections through chemical interference (Pires et al. 2003) in a way similar to allelopathy in plants. According to our results, *B. pitanga* could potentially increase the likelihood of local extinction in aquatic hosts, and over multiple generations, become the dominant host species through selection. We must highlight, however, that this final outcome of selection was not captured by our short-term experiment, as competitive exclusion could not take place during the length of our study. Nevertheless, the multiple aspects of host species interactions highlight the importance

of measuring both complementarity and selection, allowing us to propose further hypotheses about potential mechanisms for species- and community-level processes leading to pathogen dilution or amplification.

Our results, combined with previous empirical laboratory studies (Searle et al. 2011, Vanesky et al. 2013), support the DE in amphibian-*Bd* systems. In contrast, our previous field-based empirical studies found strong support for *Bd* amplification (Becker & Zamudio 2011). Using field-collected data, we reported a positive relationship between amphibian species richness and *Bd* infection, after accounting for the effects of land cover and climate (Becker & Zamudio 2011). Two habitat generalist amphibians from Costa Rica (the Rain frog, *Craugastor fitzingeri*) and Australia (the Stony Creek frog, *Litoria lesueuri*) showed higher *Bd* occurrence, prevalence, and infection loads in diverse communities. We hypothesized that natural species-rich communities are more likely to include competent hosts for *Bd* than depauperate ones, increasing pathogen spillover. For instance, natural species-rich communities include a higher proportion of stream-dwelling specialists (Becker et al. 2007) that often carry higher *Bd* infection intensities in the wild (Kriger & Hero 2007; Lips et al. 2008). Most host species in these diverse natural communities may have had a higher likelihood of suffering *Bd* spillover from the highly infected stream dwellers, such as species of *Atelopus* in Central America (Lips et al. 2008) and *Taudactylus* in Australia (Schloegel et al. 2006). Combined, these findings illustrate that studies investigating diversity-disease relationships will show contrasting results when non-additive and additive effects of diversity are not quantified independently. Because observational field studies cannot fully disentangle the impact of species interactions from additive

effects, laboratory controlled experiments will continue to be important to understand mechanisms of species interactions driving wildlife diseases.

Global biodiversity is declining sharply due in large part to anthropogenic habitat change and emerging diseases (Daszak et al. 2000, Harvell et al. 2002). Therefore, understanding the mechanisms by which biodiversity alters disease dynamics can considerably advance the field of disease ecology and has important implications for conservation of natural populations. Our results indicate that shifts in host interactions and habitat use - both mechanisms of complementarity - can drive DE. In our study system, dilution was likely driven by interactions in diverse assemblages that reduced host contact rates and *Bd* transmission. Partitioning the net effect of host diversity on disease across several unique communities, rather than relying on the correlative effects of host species richness, evenness, and composition, will allow us to identify specific mechanisms and test for their generality across host communities. Finally, our study shows that the application of methods from BEF can facilitate new avenues in experimental design and data analysis, with important theoretical implications for the field of disease ecology and practical implications for understanding and predicting wildlife epidemics.

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CHAPTER 5

HABITAT CHANGE, HOST COMMUNITY STRUCTURE, AND DISEASE RISK IN TEMPERATE AND TROPICAL AMPHIBIANS

Abstract

Habitat disturbances and the emergence of the frog killing fungus *Batrachochytrium dendrobatidis* (*Bd*) have been linked to amphibian population declines and extinctions worldwide. Deforestation can directly alter amphibian community structure through biotic and abiotic mechanisms including species interactions and local microclimates. These changes in amphibian community attributes, in turn, potentially impact *Bd* infection dynamics and thus also have an indirect role in biodiversity persistence. Here, we tested whether deforestation influences *Bd* infection intensity through shifts in amphibian species richness, community composition, total host density, and biomass. We surveyed 22 temperate and tropical amphibian communities across gradients of habitat disturbance in the U.S. and Brazil, and experimentally exposed a subsample of each amphibian host community to standardized *Bd* zoospore loads in mesocosms under controlled microclimate. We found that denser temperate amphibian communities found in pristine closed-canopy sites showed higher *Bd* loads than communities typical of disturbed environments, when microclimates were held constant. In contrast, tropical amphibian communities commonly found in pristine forests carried lower *Bd* infection loads in the absence of microclimate effects, likely due to host species composition. Our results highlight that the deforestation has cascading effects on disease risk, and that the resulting changes in amphibian community structure can lead to contrasting *Bd* outcomes in tropical and temperate amphibian communities. Quantifying the contribution of host community attributes to *Bd* infections will help us identify specific

drivers of disease and inform conservation strategies in amphibian communities in the wild.

Introduction

Amphibian biodiversity is declining worldwide at unprecedented rates (IUCN et al. 2014). Two important factors implicated in population declines and extinctions are habitat loss (Cushman 2006, Becker et al. 2007) and chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Skerratt et al. 2007, Lips et al. 2008, Wake & Vredenburg 2008, Hof et al. 2011). Loss of natural vegetation shifts amphibian community structure by increasing population isolation (Arens et al. 2007), inbreeding (Andersen et al. 2004), edge effects (Urbina-Cardona et al. 2006), and discontinuity between terrestrial and aquatic habitats (Rittenhouse & Semlitsch 2006, Becker et al. 2010). Disturbances to natural habitats also change ecosystem structure, shifting both macro (Costa & Foley 2000) and microclimates (Kapos 1989). Therefore, habitat change can have potentially large effects on amphibian susceptibility to disease, by altering host community structure and optimum microclimates for both amphibian hosts and pathogens such as *Bd* (Patz et al. 2000, Woodhams et al. 2008, Becker et al. 2012, Elderd & Reilly 2014).

Bd is a waterborne epidermal pathogen with a broad host range among amphibians (Fisher et al. 2009). Because many amphibian species share aquatic breeding sites (e.g., ponds, streams), inter-host transmission is common (Searle et al. 2011). Free-swimming zoospores shed by infected individuals colonize the skin of other host species from water (Blaustein et al. 2005) and proliferate over a few days

causing chytridiomycosis (Longcore et al. 1999). Shifts in amphibian community attributes are expected to influence *Bd* dynamics (Ostfeld & Keesing 2012, LoGiudice et al. 2003), because host species vary in their susceptibility to infection (Blaustein et al. 2005, Gervasi et al. 2013) and possibly shed zoospores into aquatic habitats at different rates (DiRenzo et al. 2014). Likewise, density-dependent transmission, where disease increases with host density (Searle et al. 2011, Venesky et al. 2014), will also cause potential changes in host-pathogen dynamics as populations and communities shrink or expand in modified habitats. Therefore, we expect that shifts in host community attributes following anthropogenic habitat alteration will have potentially large impacts on pathogen burden and disease risk.

Host community attributes such as species richness, species composition, total host density, and biomass, can be precisely quantified across amphibian communities in the wild. However, their effects on host-pathogen dynamics are often confounded by the influence of abiotic factors that independently regulate pathogen abundance or host exposure. For instance, the influence of microclimate on likelihood of amphibian infection by *Bd* is unequivocal, as mounting evidence points to direct effects of both temperature and humidity on *Bd* growth and persistence both in the wild and laboratory (Longo et al. 2010, Becker & Zamudio 2011, Raffel et al. 2011, Becker et al. 2012). As deforestation drastically changes local microclimates (Kapos 1989), along with the aforementioned host community attributes (Chapin et al. 2000), integrative field and laboratory studies of host-*Bd* dynamics are necessary if we are to understand the relative contribution of biotic and abiotic forces to disease.

Here, we tested whether anthropogenic changes to natural forest vegetation influence *Bd* infection dynamics through shifts in temperate and tropical amphibian community attributes. Specifically, we used a combination of field surveys and mesocosm experiments to address hypotheses about the potential role of habitat change in mediating disease risk through shifts in host species richness, community composition, total host density and biomass. We surveyed 22 amphibian communities in temperate and tropical landscapes and quantified the effect of deforestation on host species composition and relative abundance across sampling sites. We then experimentally exposed a representative sample of each host community to standardized *Bd* zoospore loads in mesocosms to test the effect of host community attributes on *Bd* infection dynamics under controlled microclimate conditions. Combined, our field surveys and mesocosm experimental infections can advance our knowledge of chytridiomycosis by quantifying the raw influence of host community structure on host-pathogen dynamics. Understanding how each host community attribute influences *Bd* infection intensity, and how their effects may differ in tropical and temperate amphibian communities, is critical for the development of appropriate conservation efforts in the wild.

Methods

Study sites and GIS analyses

We surveyed amphibians from ten ponds in a landscape of the Eastern Forest-Boreal Transition (Adirondack Park) of the northeastern U.S. (43° 15’ N; 74° 35’ W) and 12 ponds in a landscape of the Serra do Mar Coastal Forest in southeastern Brazil

(23° 13’ S; 45° 20’ W). We quantified natural vegetation cover for each sampling site based on high-resolution orthophotos from 2008–2009 (15 and 30 cm resolution; [USGS 2010]) for the U.S. and high-resolution satellite images from 2010 (SPOT, 2-m resolution) for Brazil. For both temperate and tropical landscapes, study sites were chosen along a gradient of natural vegetation from disturbed/open to pristine/closed-canopy vegetation cover. At each sampling site, we quantified the percentage of natural vegetation in a radius of 30 m from the edge of the pond using ArcGIS v.10 (ESRI 2013). We considered urban, pasture, agriculture, silviculture, and recreational land (e.g., golf-courses, soccer fields) as non-natural land-cover types. To avoid confounding effects of elevation and macroclimate across our sampling ponds, we explicitly chose landscapes with low climatic and topographic variability (see Becker & Zamudio 2011, Becker et al. 2012).

Host community surveys in the wild and collection of amphibians for mesocosm experiments

We conducted visual encounter surveys in ponds in the Adirondack Park during the boreal summer (June) of 2012, and in ponds in Brazil during the austral summer (November) of 2012. At each sampling pond, we surveyed amphibian communities over three consecutive nights to obtain standardized data on amphibian species composition and relative abundance for all sites in our disturbance gradients. Surveys in Brazil and in the Adirondack Park took place within a window 7 and 6 days, respectively.

Using our pond survey data, we estimated amphibian species composition and relative abundance across sampling sites, and then collected a representative subsample of individual amphibians from each pond mimicking host species richness, density, and total biomass from natural communities. We swabbed, weighed, and identified to species all amphibians captured.

Mesocosm host communities

We brought to the laboratory the representative subsamples of amphibian communities for a total of 10 temperate and 12 tropical mesocosm communities. This experimental design allowed us to match host community attributes observed in the wild (i.e., see detailed list of attributes below) to mesocosm communities in the laboratory. Each experimental unit consisted of a large water tank (120cm diameter x 100cm height) with terrestrial habitat covering one half of the container (i.e., autoclaved moist *Sphagnum*) and aquatic habitat on the other half. We covered each experimental unit with plastic mesh to prevent escape of the frogs. We maintained all tanks indoors at a constant room temperature of 20˚C to control for microclimatic variation. Our temperate amphibian communities had three individuals minimum and 20 individuals maximum per experimental tank (Table 5.1), whereas our tropical amphibian communities had two individuals minimum and 29 individuals maximum per experimental tank (Table 5.2). We included a total of five temperate amphibian species and 14 tropical amphibian species in our experiments.

Table 5.1: Host species composition and total abundance in our ten temperate mesocosm enclosures. AI stands for aquatic index; habitat loss (%) for sampling sites is given.

Species	Family		*Temperate sampling ponds*										Total
		AI	*P1*	*P2*	*P3*	*P4*	*P5*	*P6*	*P7*	*P8*	*P9*	*P10*	
Anaxyrus americanus	Bufonidae	*2*							1			1	4
Lithobates catesbeianus	Ranidae	*2*	1					3					6
Lithobates clamitans	Ranidae	*2*	3	9	3	13	2	2	17	12	7	6	76
Pseudacris crucifer	Hylidae	*1*	1	1		1			2		2	1	9
Lithobates palustris	Ranidae	*2*			1		1						4
Total			5	10	4	14	3	5	20	12	9	8	90
Habitat Loss (%)			49.13	15.29	67.15	21.31	87.06	88.83	5.27	98.66	46.85	98.39	

Table 5.2: Host species composition and total abundance in our twelve tropical mesocosm enclosures. AI stands for aquatic index; habitat loss (%) for sampling sites is given.

Species	Family	AI	*Tropical sampling ponds*												Total
			P1	*P2*	*P3*	*P4*	*P5*	*P6*	*P7*	*P8*	*P9*	*P10*	*P11*	*P12*	
Rhinella icterica	Bufonidae	*2*		2					1			2	5	1	11
Rhinella ornata	Bufonidae	*2*									1		1		2
Proceratophrys boiei	Cycloramphidae	*2*						1							1
Dendropsophus microps	Hylidae	*1*			5	3									8
Dendropsophus minutus	Hylidae	*1*	2		8	20	1								31
Hypsiboas albopunctatus	Hylidae	*1*								1					1
Hypsiboas bandeirante	Hylidae	*1*	1		5		2	1							9
Hypsiboas faber	Hylidae	*1*	2			2	3								7
Hypsiboas pardalis	Hylidae	*1*					1				1				2
Hypsiboas prasinus	Hylidae	*1*											2		2
Scinax crospedospilus	Hylidae	*1*	2					1		3					6
Scinax Hayii	Hylidae	*1*	3		2	4		5							14
Physalaemus cuvieri	Leptodactylidae	*2*					3			3					6
Lithobates catesbeianus	Ranidae	*2*		1					1			1		1	4
Total			10	3	20	29	10	8	2	7	2	3	8	2	105
Habitat Loss (%)			58.9	85.0	19.5	19.8	26.6	37.8	62.9	91.7	45.1	90.3	100	75.1	

Host community attributes in mesocosms

We define host species richness as the number of amphibian species in each experimental unit. As *Bd* infects all host species used in this study (Becker & Zamudio 2011, Gruendler et al. 2012), we quantified total host density as the total number of individual amphibians within each experimental unit. Similarly, we measured biomass as the sum of body mass across all individual hosts within each experimental unit. In addition to these community attributes, we measured two variables (community similarity and host aquatic index), that provide quantitative data on the similarity among sampled communities in terms of species composition and life-histories influencing the likelihood of *Bd* infection at the level of community. The first variable measures similarity across communities. We consolidated data on species composition and relative abundance across sampling ponds using Non-Metric Multidimensional Scaling (NMDS) and used the first axis NMDS as a metric of community similarity. Ecologically similar communities, in terms of species composition and relative abundance, have similar NMDS values, and therefore, axis 1 of NMDS is a proxy of how similar communities are in terms of host identity and relative abundance. We chose this method over traditional similarity indices (i.e., Sorensen, Bray-Curtis) because our analytical design cannot be based on pairwise species relative abundance data. The second variable is an index for which each species uses aquatic habitats during lifetime (aquatic index; AI; modified from Lips et al. 2003), thus varying in their degree of *Bd* exposure and transmission (Lips et al. 2003). Thus, arboreal species (i.e., Hylidae) that spend less time in water were coded AI=1 and species occupying aquatic vegetation and terrestrial stratum around ponds (i.e., Bufonidae,

Leptodactylidae, Cycloramphidae, Ranidae) were coded as AI=2. We then averaged AI across all individuals in each experimental unit, reaching a weighed average AI by species for each community. This classification resulted in a gradient across sampling sites with low average AI communities dominated by arboreal pond-breeding species, and high average AI communities dominated by riparian/terrestrial pond-breeding species.

Experimental infections

Prior to the experimental infections with temperate amphibians, we cleared any *Bd* field-infected animals in the laboratory by keeping animals housed at a temperature of 31°C for 12 consecutive days and changing the water every three days (Woodhams et al. 2003, Forrest & Schlaepfer 2011). Prior to the experimental infections in Brazil, we administrated a 7-day disinfection treatment using Itraconazole at 0.01% solution (Pessier & Mendelson 2010). In both cases, we verified clearance of pathogens from naturally infected individuals using qPCR.

For experimental infections, we inoculated frogs using locally-isolated *Bd* strains. We cultured global panzootic *Bd* strains JEL404 (from Maine, U.S.) and CLFT023 (from Minas Gerais, Brazil) at 19°C for 7d. We harvested *Bd* by flooding plates with distilled water and waiting ~3 h for zoospore release. We then pooled inoculum from plates, quantified zoospores with a hemocytometer, and added 10^7 zoospores in 6L of dechlorinated water to the water in each experimental unit. This protocol guaranteed comparable infection regimes across replicates, with *Bd* strains naturally experienced by the hosts in each community. We added *Bd*-negative

amphibians to the terrestrial habitat of each experimental unit and kept temperatures at 20°C on a 12 h day-night light cycle. We monitored amphibians daily and fed them pinhead crickets and wingless flies *ad libitum*.

We swabbed all individuals prior to the experimental infection and upon termination of the experiment on the 18th day post-infection. This period encompasses approximately five replication cycles of the pathogen (Longcore et al. 1999) and is sufficient for *Bd* to reach peak infections in susceptible captive amphibians (Longo et al. 2010, Savage & Zamudio 2011). In case of disease, we swabbed dead or dying animals and removed them from the experimental units. We tested swabs for *Bd* using Taqman qPCR (Boyle et al. 2004, Hyatt et al. 2007) with standards of 0.1, 1, 10, 100, and 1000 zoospore genomic equivalents (GE) to determine the infection intensity of *Bd* in each sample. At the end of the experiment, we euthanized all animals using Benzocaine 4%. All experimental procedures were approved by Cornell IACUC # 2010-0069.

Statistical Analyses

We quantified the effect of percent natural vegetation surrounding sampling ponds on each host community attribute using Standard Least Square General Linear Models (GLMs). We then employed GLM model selection entering host community attributes as biological explanatory variables, including one-level interactions, to find the combinations of variables that best explained *Bd* infection intensity ($\log_{10}$ transformed) at the end of the experiment. In addition to our host community metrics, we included community-level data on average *Bd* infection intensity observed in the

wild as a control variable for previous exposure and potential acquired resistance. Competing models were ranked based on Akaike Information Criterion (AIC), and we reported the model with the highest goodness-of-fit for each run. We did not analyze prevalence data because >96% of the hosts became infected with *Bd* during the experiments and because infection intensity, measured as mean pathogen load in the community, is a better indicator of infection dynamics in mixed populations. Mortality was negligible across tropical amphibian communities (i.e., only a single frog died) and thus we only analyzed mortality for temperate communities. We performed all analyses using JMP v. 10.0 (SAS 2010) and SPSS (IBM Corp. 2013).

Results

In temperate amphibian communities, we found marginally higher host capture rates in ponds surrounded by natural vegetation than in ponds in disturbed habitats ($F_{[1,8]} = 4.826$, $\beta = 0.091$, $P = 0.059$), but the amount of natural vegetation did not predict host species richness, community similarity, average AI, and biomass. Temperate host communities were dominated by *L. clamitans* across the gradient of habitat alteration. The observed higher amphibian capture rate in ponds surrounded natural habitats – a proxy for higher host densities – predicted higher average *Bd* infection intensity in amphibian communities under controlled microclimate conditions in the laboratory ($F_{[1,8]} = 6.410$, $\beta = 0.067$, $P = 0.035$; Figure 5.1). In addition to elevated *Bd* infection intensity, communities with high host density showed a non-significant trend of higher mortality rates during the course of the experiment ($F_{[1,8]} = 2.005$, $\beta = 0.172$, $P = 0.194$).

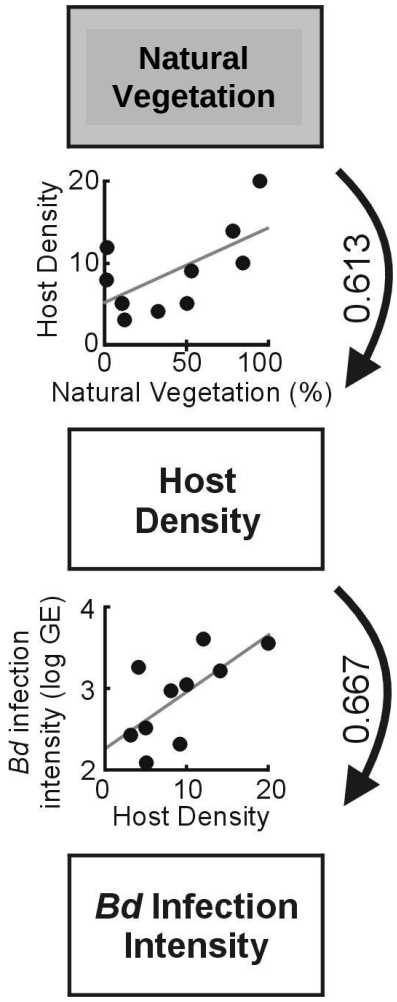

Figure 5.1: Unidirectional causal relationship between natural vegetation surrounding temperate ponds in the Adirondacks, total host density, and average *Bd* infection intensities across mesocosm amphibian communities. Linear regressions are shown for each relationship; arrows show standardized betas.

Looking simultaneously at all explanatory biological variables controlling *Bd* infection intensity in our mesocosm communities through GLM model selection, the most parsimonious model included total host density as the single positive predictor of *Bd*. These findings were not associated with host acquired resistance, as community-level *Bd* infection loads observed in the laboratory were unrelated to respective loads observed in the wild prior to the experiment ($F_{[1,8]} = 0.069$, $P = 0.798$).

In our diverse tropical communities from Brazil, we found the same pattern of higher host capture rates in ponds surrounded by natural vegetation ($F_{[1,10]} = 7.208$, $\beta = 0.181$, $P = 0.023$). In these communities, we also found a significant positive effect of natural vegetation on host species richness ($F_{[1,10]} = 5.469$, $\beta = 0.024$, $P = 0.041$) and a significant effect on community similarity ($F_{[1,10]} = 10.418$, $\beta = 0.028$, $P = 0.009$). None of the explanatory biological variables we measured significantly predicted *Bd* loads in our mesocosm experimental infection when regressed independently. However, model selection quantifying simultaneously all variables potentially explaining *Bd* infection intensity identified three important host community attributes: community similarity, average AI, and *Bd* infection loads observed in the wild (Table 5.3). Specifically, assemblages of species from disturbed habitats showed higher *Bd* infection loads following experimental infection in the laboratory, and this pattern held even after controlling for the effect of previous exposure to *Bd*. Communities dominated by arboreal amphibian species (Hylidae, AI=1) also showed higher *Bd* infection intensities than communities dominated by riparian/terrestrial species (AI=2).

Table 5.3: Generalized Linear Model (GLM) simultaneously testing the effects of host community similarity, average AI, and previous host infection in the wild on *Bd* infection intensity across tropical amphibian communities in Brazil, under controlled microclimates in the laboratory. Variance inflation factor (VIF) denotes colinearity in the model when higher than 10.

Variable	**Std *Beta***	***t* Ratio**	**VIF**	***P***
Intercept		*6.21*	*.*	*<0.001*
Community similarity (NMDS axis I)		2.75	2.725	0.025
Average host aquatic index (AI)	-0.795	-2.62	2.880	0.030
Bd infection loads in the wild (logGE)	-0.531	-2.8	1.133	0.023

Full model statistics: $F_{[8,3]} = 7.784$, $r^2 = 0.745$, $P = 0.009$.

Discussion

The mechanisms controlling disease dynamics in wildlife can be affected by a myriad of biotic and abiotic factors (Ostfeld & Keesing 2012). Our field and laboratory experiments confirmed our predictions that shifts in host community attributes arising from changes in natural vegetation play an important role in amphibian host-pathogen interactions, independent of the well-known influence of microclimate (Longo et al. 2010, Becker & Zamudio 2011, Raffel et al. 2011, Becker et al. 2012). Our results show that amphibian communities commonly found in pristine tropical Brazilian forests carry lower *Bd* infection loads when abiotic forces are maintained constant, presumably due to their host species composition. In contrast, our results indicate that denser temperate amphibian communities from pristine closed-canopy sites are associated with elevated *Bd* loads under constant microclimate, supporting previous findings of higher disease risk in natural habitats in the northeastern U.S. (Becker et al. 2012). Our combined results highlight that disturbances to natural vegetation can either increase or decrease *Bd* infection loads in amphibians through shifts in host community attributes, independent of the well-recognized effects of variable microclimate (Longo et al. 2010, Becker & Zamudio 2011, Raffel et al. 2011, Becker et al. 2012).

Tropical Amphibian Communities from Disturbed Habitats Show Increased Disease Risk

Deforestation raises local temperatures (Kapos 1989), which can be enough to suppress *Bd* growth and persistence in open disturbed habitats (Raffel et al. 2011, Forrest & Schlaepfer 2011, Becker et al. 2012). However, the impact of microclimate

on *Bd* infection loads is expected to vary across space and time (Kriger et al. 2007, Lenker et al. 2014), and thus interact with host community attributes synergistically or antagonistically. Deforestation in southeastern Brazil significantly modified host community composition and disease risk, such that tropical amphibian species commonly found in disturbed habitats carried higher *Bd* infection loads in the laboratory. One potential explanation is the presence of the exotic American bullfrog (*Lithobates catesbeianus*) − a species introduced into South America in the early 1900s (Schloegel et al. 2012) – which is present in disturbed landscapes across the Atlantic coastal forest, including in our landscape (Giovanelli et al. 2008). As bullfrogs carry high *Bd* infection loads without signs of disease (Schloegel et al. 2012), their presence can boost average community infection loads. Furthermore, they can serve as pathogen reservoirs and increase the risk of disease in other amphibian species occupying disturbed habitats. Similarly, highly competent amphibian hosts for the parasitic trematode *Ribeiroia ondatrae* dominate species-poor communities, increasing disease risk in altered landscapes (Johnson et al. 2013). Although we did not measure this in our system, decreases in host genetic diversity as a result of deforestation is another potential candidate mechanism to explain the observed pattern. A recent laboratory study found a negative association between host genetic diversity and *Bd* infection loads in populations of a North American frog (Savage & Zamudio 2011).

We also hypothesized that changes in host species richness due to habitat alteration could significantly influence host-pathogen interactions (Patz et al. 2000, Schmidt & Ostfeld 2001), as high host diversity often reduces pathogen burden; a mechanism known as the dilution effect (Keesing et al. 2006). Recent studies reported

a dilution effect in host-*Bd* systems, such that an increased number of amphibian species reduced infection loads in tadpoles (Searle et al. 2011; Venesky et al. 2014) and post-metamorphic amphibians (see Chapter 4). Therefore, we hypothesized that our mesocosm communities with high host diversity would show lower *Bd* infection loads. However, total host density and species richness were positively correlated. Communities with high amphibian density, which should carry average higher *Bd* infection loads, also showed elevated diversity, which presumably decreases the odds of infection. Therefore, it is possible that density-dependent transmission and the effect of host diversity have canceled each other out in our experiment with tropical amphibian species.

Density-dependent Transmission in Temperate Amphibians

If host communities do not vary significantly in species composition, diversity, or genetic factors to resist infection, then pathogen build-up towards high infection intensities is more likely to occur in denser host populations under conditions that promote continuous reinfection of hosts (Briggs et al. 2010). Laboratory studies show that *Bd* transmission rates in temperate amphibians increase with the density of infected hosts (Rachowicz & Briggs 2007, Searle et al. 2011, Venesky et al. 2014). Furthermore, our previous work showed that in natural amphibian communities host density-dependent forces likely control *Bd* infection loads in the wild, though the small-scale effects of microclimate on host-*Bd* systems are stronger (Becker et al. 2011, Becker et al 2012). Specifically, host density positively predicted *Bd* infection intensity in wild-caught *L. clamitans* when analyzed independently in a simple linear

regression, but its effect became non-significant when considered together with other environmental factors such as water temperature and degree of shade at natural sites (Becker et al. 2012). Still, amphibians in ponds surrounded by natural vegetation, such as in the Adirondack region, are expected to carry higher *Bd* loads due to both higher host densities and suitable temperatures for *Bd*, which presents a conundrum for amphibian conservation.

Conclusions

With the high rate of anthropogenic modification affecting temperate and tropical forests (Chapin et al. 2000), understanding how deforestation influences disease risk is critical for predicting *Bd* spread and developing appropriate management tools for wild populations. The present study is the first to employ challenge infection experiments aiming at comparing disease risk in representative amphibian assemblages in terms of species composition and relative abundance. Our results highlight that deforestation can lead to an increase or decrease in amphibian disease risk depending on how habitat disturbances change host community structure. Therefore, quantifying the net contribution of host community structure to chytridiomycosis in regions experiencing amphibian declines will help us identify case-specific drivers of disease in the age of anthropogenic habitat destruction and global pandemics.

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APPENDIX

THE BRAZILIAN ADIRONDACKS?

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Letter to the editor

The name of the Serra da Mantiqueira Mountains, translated from the indigenous Tupí-Guaraní language as "Weeping Mountains," underscores their historical importance as a source of water for southeastern Brazil. Mantiqueira harbors outstanding sociocultural history, and its high peaks form a key wildlife corridor within the Atlantic Forest Biodiversity Hotspot (1). The striking scenery and proximity to Rio de Janeiro and São Paulo (17.6 million inhabitants combined) offer an opportunity to consider new models in conservation and sustainability.

In 2006, the Brazilian Ministry of the Environment created the Mantiqueira Mosaic, a network of public and private conservation units, to enhance biological conservation and local welfare. However, the implementation of most conservation units has been delayed and many still lack a management plan. For instance, the creation of the largest park (Parque Nacional Altos da Mantiqueira), which will cover 86,000 hectares of high peaks (2), has been stalled since 2010. Brazil's booming economy, and the 40% predicted increase in agricultural land use in the next decade (3), will pose serious threats to the remaining ~10% of the Brazilian Atlantic Forest (4).

In the 19th century, the Adirondack Mountains, adjacent to New York City and Albany (8.4 million inhabitants), faced a strikingly similar scenario due to logging and mining activities, leading to creation of the Adirondack Park in 1892 (5). Along with the Algonquin Provincial Park in Canada, the Adirondacks serve as a key wildlife corridor in North America (6). The Adirondack Park successfully manages an

integrated mosaic of private and publicly owned lands, supports sustainable land use, regulates recreational activities, and promotes ecotourism and education (5).

The conservation challenges for the Mantiqueira range and Adirondacks are similar, despite being proposed more than a century apart. The future of Brazil's most prominent mountain chain as wilderness now depends on accelerated implementation of the conservation network by the Brazilian Federal Government so that the Mantiqueira Mosaic can promote biodiversity conservation and environmental stewardship through sustainable land use and citizen access.

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SUPPLEMENTARY INFORMATION

Table S2.1: Model selection for biotic and abiotic variables influencing *Bd* occurrence in populations of *C. fitzingeri* in Costa Rica; and *Bd* prevalence and infection intensity in populations of *L. lesueuri* in Australia. We compared all possible models using Akaike Information Criterion (AIC); five best models are reported for each dataset.

OCCURRENCE

Model	AIC	nVars
LOSS, RICH	67.584	2
LOSS, RICH, BIO7	67.974	3
LOSS, RICH, BIO7, RICH*BIO7	68.891	4
LOSS, RICH, BIO7, RICH*BIO7, LOSS*BIO7	68.918	5
LOSS, RICH, LOSS*RICH	69.166	3

PREVALENCE

Model	AIC	nVars	r^2
LOSS, RICH, LAT&BIO5, RICH*LAT&BIO5	271.594	4	0.632
LOSS, LAT&BIO5, BIO18	273.882	3	0.590
LOSS, RICH, LAT&BIO5, BIO18, RICH*LAT&BIO5	274.516	5	0.641
LOSS, RICH, LAT&BIO5, RICH*LOSS, RICH*LAT&BIO5	274.671	5	0.639
LOSS, RICH, LAT&BIO5, LOSS*LAT&BIO5, RICH*LAT&BIO5	275.290	5	0.632

INFECTION INTENSITY

Model	AIC	nVars	r^2
LOSS, BIO14	89.429	2	0.582
LOSS, BIO14, LOSS*BIO14	91.892	3	0.590
LOSS, LAT&BIO8, BIO14	92.467	3	0.582
LOSS, LAT&BIO8, BIO14, LOSS*LAT&BIO8	93.954	4	0.607
LOSS, LAT&BIO8, BIO14, LOSS*BIO14	95.188	4	0.591

Best predictors are: habitat loss (LOSS), amphibian species richness (RICH), temperature annual range (BIO7), precipitation of warmest quarter (BIO18), latitude and maximum temperature of the warmest month consolidated in the first principal component [96.00% of the variation in the original variables (LAT&BIO5)], latitude and mean temperature of wettest quarter consolidated in the first principal component [95.40% of the variation in the original variables (LAT&BIO8)], and precipitation of the driest month (BIO14).

Table S3.1: Model selection for environmental and biological variables influencing *Batrachochytrium dendrobatidis* (*Bd*) prevalence and infection intensity in populations of *Lithobates clamitans* in the Adirondack region, New York, USA.

Model	**AICc**	**nVars**
Prevalence		
CANOPY	75.607	1
CANOPY, MAXTEMP	81.512	2
MAXTEMP	82.334	1
AVTEMP	83.047	1
CANOPY, AVTEMP	83.666	2
Infection Intensity		
AVTEMP, NATVEG, AVTEMP*NATVEG	-17.690	3
CANOPY	-10.657	1
CANOPY, DIVERSITY	-5.951	2
CANOPY, MAXTEMP	-3.032	2
CANOPY, AVTEMP	-2.509	2

We ranked all possible models using Akaike Information Criterion (AICc); five best models are reported for each dataset. Best predictors are: canopy density (CANOPY), natural vegetation (NATVEG), water temperature - daily average (AVTEMP), water temperature - average maximum (MAXTEMP), and host community diversity (DIVERSITY).

Table S4.1: Non-additive effects of host diversity on *Bd* infection loads. Mean net effect, complementarity and selection across diverse assemblages, excluding mixtures that experienced mortality (N=6).

Factor	Mean	Upper 95% CI	Lower 95% CI
Net effect of host diversity	-0.863*	-0.096	-1.630
Complementarity	-0.839*	-0.050	-1.628
Selection	-0.024	0.142	-0.191

** P < 0.05; N=19.*